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<b>(21) International Application Number:</b> PCT/EP96/02311 <b>(22) International Filing Date:</b> 31 May 1996 (31.05.96) <b>(30) Priority Data:</b> 9510944.3 31 May 1995 (31.05.95) <b>GB</b> <b>(71)(72) Applicants and Inventors:</b> BOGAERT, Thierry [BE/BE]; Voorstraat 36 bus 11, B-8500 Kortrijk (BE). STRINGHAM, Eve [CA/CA]; 9326-133 A Street, Surrey, British Columbia V3V 5R5 (CA). VANDEKERCKHOVE, Joel [BE/BE]; Rode Beukendreef 27, B-Loppem (BE). <b>(74) Agent:</b> BALDOCK, Sharon, Claire; Boulton Wade Tennant, 27 Fumival Street, London EC4A 1PQ (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> PROCESSES FOR THE IDENTIFICATION OF COMPOUNDS WHICH CONTROL CELL BEHAVIOUR, THE COMPOUNDS IDENTIFIED AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND THEIR USE IN THE CONTROL OF CELL BEHAVIOUR  <b>(57) Abstract</b>  UNC-53 protein of <i>C. elegans</i> or its functional equivalent is identified as a signal transducer/integrator involved in controlling the rate and directionality of cell migration and/or cell shape. Nucleic acid sequences encoding UNC-53 protein or its functional equivalent, such as genomic or cDNA are used to transfect <i>C. elegans</i> or mammalian cell lines useful for identifying inhibitors or enhancers of the UNC-53 protein. Any of the inhibitors or enhancers identified or the UNC-53 protein itself or sequences encoding UNC-53 protein can be used in the preparation of medicament for treatment of neurological conditions such as Alzheimer's or Huntingdon's disease, peripheral neuropathies for inhibition of metastasis.		

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PROCESSES FOR THE IDENTIFICATION OF COMPOUNDS  
WHICH CONTROL CELL BEHAVIOUR, THE COMPOUNDS IDENTIFIED  
AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND  
THEIR USE IN THE CONTROL OF CELL BEHAVIOUR

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The present invention relates to processes for the identification of compounds which inhibit or enhance the rate and direction of cell migration or the control of cell shape, the compounds identified and pharmaceutical formulations containing such compounds together with their use in the regulation of cell behaviour. The invention also relates to an UNC-53 protein encoded by nucleic acid in the cells of the nematode worm C. elegans and cDNA sequences encoding an UNC-53 protein or functional equivalents thereof.

15

The control of cell motility, cell shape and the outgrowth of axones or other cell outgrowths is an essential feature in the morphogenesis and function of both unicellular and multicellular organisms. The control of this process is disturbed in a variety of disease states in which for example the Receptor Tyrosine Kinase (RTK) signal transduction pathways or the like or their downstream intra-cellular pathways (which are shared with other extra-cellular receptors, including cell adhesion molecules like N-CAMS and integrins) are overstimulated.

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Some cell surface proteins and extracellular molecules controlling the directionality and potential of cell migration have been identified. However the processes in which these proteins or molecules are involved to effect cell migration, shape or rate of cell differentiation are not understood.

30

It is generally considered that a long-range migration of a cell process (which may also be known as a growth cone spike) is a stepwise event, whereby prior to and after each extension there is the

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formation of a structure at the leading edge of the cell which senses signals in the environment instructing the cell to either stabilize a cell process extending in a preferred direction, or to  
5 cause a cell process lamellipodium to extend a process in a given direction. Localized stabilization of the actin cytoskeleton, is a general cell biological process underlying this choice of directional extension.

10 A gene from the free-living nematode Caenorhabditis elegans, designated "unc-53" has been previously identified and cloned (Abstract, International C. elegans meeting; June 1-5 1991, Madison, Wisconsin, 58, Bogaert and Goh). However, to  
15 date no known biological function has been attributed to the unc-53 gene or its corresponding UNC-53 protein.

The present inventors have surprisingly identified, through biochemical, genetic, phenotypic  
20 and transgenic evidence which is presented herewith, UNC-53 as a signal transducer or signal integrator controlling the rate and directionality of cell migration, and/or cell shape. Key experiments leading to this conclusion were the molecular identification  
25 of its domain structure, its biochemical interaction with GRB-2, actin cytoskeleton sequence information and the presence of a potential signal integrating domain in the UNC-53 protein.

An additional key observation is that increased  
30 UNC-53 protein activity is proportional to increased cell process extension in the correct direction of cell migration. Reduction of UNC-53 function has previously been shown to lead to a reduction of cell process extension, identifying it as a general  
35 component required for cell migration. However, it had not been identified as a component whose level of



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activity has a determining role in the specification of the quantum and directionality of migration.

The work of the present inventors suggests that UNC-53 plays a central role in quantitatively transducing extracellular signals to the machinery controlling directional cell migration.

The importance of UNC-53 in a variety of cell types in C. elegans has been demonstrated. The gene encodes a signal transduction molecule that transduces a signal from a Receptor Tyrosine Kinase such as for example via the adaptor protein SEM-5/GRB-2, to the machinery controlling directional growth cone extension or stabilization. The UNC-53 protein does this in a highly dosage-dependent fashion whereby reduction of protein activity such as reduction in expression of protein or in the reduction in its activity leads to proportional reduction of cell process extension (cell migration). This is believed to be either by regulated cross-linking of the actin cytoskeleton or by transferring the received signal downstream within the transduction pathway. Higher than wild type UNC-53 expression leads to higher than wild type growth cone extension in the anterior-posterior axis. Both the observed SEM-5/GRB-2 binding to UNC-53 and the predicted ATP/GTP-ase activity of UNC-53 demonstrate a signal transduction role for UNC-53 involved in cell process or growth cone guidance.

UNC-53 is a protein working at the intracellular level. It is so far believed to be the only intracellular protein identified which is involved in the control of directionality and rate of cell migration in response to a specific signal and which integrates different directional signals in defining direction of migration.

Based on the present inventors accumulated

knowledge of the unc-53 gene function in C. elegans it is understood that inhibitors or enhancers of the unc-53 gene or the UNC-53 protein will affect the cell motility including (metastasis) via an RTK pathway or the like, or may lead to changes in the shape of the cells (which has been demonstrated in C. elegans body muscle). Applications for such inhibitors and/or enhancers are envisaged in a wide variety of pathologies in which the RTK pathways play a central role, including oncogenesis, psoriasis, cell migration (metastasis), neuronal regeneration/degeneration and immunological disorders among others.

The identification of the biochemical function of the unc-53 gene (and UNC-53 pathway) in the RTK signal transduction pathway is novel and unexpected. No biological function has previously been linked to the unc-53 gene or UNC-53 protein, nor has any homology with any other nucleic acid sequence or gene been recognised.

An analysis of the predicted protein sequence of UNC-53 from the gene sequence thereof has revealed the following:

- (a) an N-terminal domain with homology to cortical actin binding proteins of the  $\alpha$ -actinin and  $\beta$ -spectrin families (designated ABPII in Figure 11). Alignment of UNC-53 with the  $\alpha$ -actinin and  $\beta$ -spectrin family of proteins is shown in Fig. 15.).
- (b) two putative actin binding sites of the LKK class (ABS1 and ABS2).
- (c) two polyproline rich sequences similar to the SH3 binding domains of the SOS family of signal transduction molecules (SH3 binding site) (Fig. 16).
- (d) a putative ATP/GTP nucleotide binding site having some of the additional features of the GTP

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binding domain of RAS-like proteins (Dynamin, NBD).

5 (e) besides the N-terminal region of the protein, which is similar to actin binding proteins, the predicted protein sequence of UNC-53 identified two putative actin binding sites. The first borders on the 3' end of the region of  $\alpha$ -actinin/ $\beta$ -spectrin homology and the second lies in the 3' end of the cDNA sequence.

10 This suggests that UNC-53 could potentially bind two actin molecules and via actin cross linking, could stabilize a particular cell process to promote directional extension.

15 In addition, genetic evidence shows that alleles of unc-53 enhance the sex myoblast migration defect of sem-5 mutants. Sem-5 represents the C. elegans homologue of GRB2, the function of these proteins being assigned/attributed to their SH2 and SH3 domains (Clark et al., (1992) Nature 356, 340-344; Stern et al., (1993), Molec. Biol. Cell, 4, 1175-1188). The  
20 current model regarding sem-5 function in the migration of sex myoblasts is that sem-5 transduces a signal received at the cell surface by egl-15, a receptor kinase of the fibroblast growth factor  
25 family. Together, the genetic and molecular data suggest a role for UNC-53 in both signal transduction and actin binding. We have been able to demonstrate how UNC-53 might act to direct both growth cone rate and directionality. By binding directly to the actin  
30 cytoskeleton, UNC-53 may stabilize and cross-link actin molecules (assuming a two actin binding site model) to promote directional growth cone extension. Alternatively, by binding actin, UNC-53 may convey a signal to the cytoskeleton and then via an ATP/GTPase  
35 activity transduce the signal to downstream targets. To test these models, biochemical experiments were

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conducted to determine if any of the sequence similarities observed represented functional domains (see examples 2 to 5). Transgenic analysis as described in examples 6 to 8 support this proposed model.

As described above, the unc-53 gene from C. elegans has been previously identified. However, cDNA sequences substantially corresponding to unc-53 genomic exon sequences of C. elegans or fragments or derivatives thereof have never been previously disclosed. The present inventors have advantageously identified two unc-53 cDNA clones which have been designated as the 7A and 8A clones. The two clones differ in the number of Adenosine(A) residues (7 or 8) in a poly A stretch of the 3' coding region. Therefore, the two clones have different reading frames in the carboxyterminal coding region.

Therefore according to one aspect of the present invention there is provided a cDNA encoding an UNC-53 protein of C. elegans or a functional equivalent derivative or bioprecursor of said protein which cDNA comprises at least from nucleotide position 431 to nucleotide position 4647 or alternatively to the 3' poly-A region of the sequence shown in Figure 1. More preferably the cDNA comprises at least from nucleotide position 64 to nucleotide position 4647 or to the 3' poly-A region of the sequence as shown in Figure 1. This cDNA is comprised in the 8A clone having 8A residues in a poly A stretch of the 3' coding region as shown in Figure 1.

In an alternative embodiment of this aspect of the invention the cDNA comprises at least from nucleotide position 431 to nucleotide position 4812 or alternatively to the 3' poly-A region of the sequence shown in Figure 2 and more preferably at least from position 64 to nucleotide position 4812 or the 3'

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poly-A region of the sequence shown in Figure 2. This  
cDNA according to the invention comprises the 7A  
clone, having only 7 Adenine residues in the poly A  
stretch of the 3' coding region as shown in the  
5 nucleotide sequence of Figure 2 page 8. Each of the  
cDNA clones according to the invention, may be  
included in an expression vector which vector may  
itself be used to transform or transfect a host cell  
which may be bacterial, animal or plant in origin.  
10 Thus, advantageously, once the cDNA corresponding to  
the unc-53 genome is synthesised using for example  
reverse transcriptase or the like, a range of cells,  
tissues or organisms may be transfected following  
incorporation of the selected cDNA clone into an  
15 appropriate expression vector.

The present invention therefore, also further  
comprises a transgenic cell, tissue or organism  
comprising a transgene capable of expressing UNC-53  
protein of C. elegans or a functional equivalent,  
20 fragment, derivative or bioprecursor thereof. The  
term "transgene capable of expressing UNC-53 protein"  
as used herein means a suitable nucleic acid sequence  
which leads to the expression of an UNC-53 protein  
having the same function and/or activity. The  
25 transgene may include for example genomic nucleic acid  
isolated from C. elegans or synthetic nucleic acid or  
alternatively any of the cDNA clones as described  
above.

The term "transgenic organism, tissue or cell" as  
30 used herein means any suitable organism and/or part of  
an organism, tissue or cell that contains exogenous  
nucleic acid either stably integrated in the genome or  
in an extra chromosomal state.

Preferably, the transgenic cell comprises either  
35 a C. elegans cell, an N4 neuroblastoma cell or an MCF-  
7 breast carcinoma cell. The transgenic organism may

be C. elegans itself, or alternatively may be an insect, a non-human animal or a plant. Preferably the unc-53 transgene comprises the unc-53 gene or a functional fragment thereof. The term "functional  
5 fragment" as used herein should be taken to mean a fragment of an UNC-53 gene which encodes an UNC-53 protein or a functional equivalent or bioprecursor of the protein. For example the gene may comprise deletions or mutations but may still encode a  
10 functional UNC-53 protein.

Reference to "tissue or tissue culture" for the purpose of the present invention should be taken to mean such a mutant cell which has been grown in such a culture. Further provided by the present invention is  
15 a mutant C. elegans organism which comprises an induced mutation, such as a point mutation in the wild-type unc-53 gene and which mutation affects the regulation of cell motility or shape or the direction of cell migration. Such mutations may be introduced  
20 using changes in the cDNA corresponding to qualitative, quantitative direct and indirect changes in the genomic make up.

The term "mutant organism" used herein means any suitable organism that contains genetic information  
25 which has been induced to mutate and is thus altered from the wild-type. Therefore naturally occurring mutations in the wild-type organism are not within the scope of this term.

The present invention further comprises an UNC-53  
30 protein or a functional equivalent or fragment thereof, which protein may be encoded by a cDNA according to the invention, and which protein has the amino acid sequence shown in Figure 4 from amino acid position 135 to amino acid position 1528; this  
35 corresponds to the 8A clone. More preferably the UNC-53 protein, when encoded by a cDNA according to the

invention, comprises the amino acid sequence shown in Figure 4. In another aspect of the invention the protein comprises an UNC-53 protein or a functional equivalent, fragment or bioprecursor of the protein which comprises the sequence of from amino acid position 135 to amino acid position 1583 of the amino acid sequence shown in Figure 6. Preferably, the UNC-53 protein when encoded by a cDNA in accordance with the invention has the amino acid sequence shown in Figure 6.

The UNC-53 protein of C. elegans or a functional equivalent, fragment or bioprecursor of the UNC-53 protein, may advantageously be used as a medicament to promote neuronal regeneration, revascularisation or wound healing or the treatment of chronic neuro-degenerative disorders or acute traumatic injuries. Similarly, the UNC-53 protein produced by the transgenic cells, tissue or organisms according to the invention may also be used in the preparation of a medicament for treatment of the conditions as described above.

Furthermore, in an alternative embodiment of the invention the nucleic acid sequence itself encoding an UNC-53 protein of C. elegans or a functional equivalent, fragment or bioprecursor of the protein may also be used as a medicament or, alternatively in the preparation of a medicament, to promote neuronal regeneration, vascularisation or wound healing or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries. Typically neurological conditions which may be treated by either an UNC-53 protein or a functional equivalent thereof, or a nucleic acid according to the invention, comprise peripheral nerve regeneration after trauma; recovery of function of the spinal cord after spinal cord trauma or peripheral neuropathies. Similarly neuro-

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degeneration diseases which may be treated include Alzheimers disease or Huntingdons disease. Acute traumatic injuries such as stroke, head trauma or haemorrhages may also advantageously be treated.

5           The nucleic acid sequence according to the invention may comprise a cDNA sequence according to the invention as described above or alternatively may be genomic DNA derived from C. elegans.

10           The UNC-53 protein of C. elegans, or a functional equivalent, fragment or bioprecursor of said protein may be incorporated into a pharmaceutically acceptable composition together with a suitable carrier, diluent or an excipient therefor. The pharmaceutical  
15           composition may advantageously comprise, additionally or alternatively to the UNC-53 protein according to the invention, the nucleic acid sequence according to the invention as defined above.

20           The present invention also provides for a method of determining whether a compound is an inhibitor or an enhancer of the regulation of cell shape or motility or the direction of cell migration in a transgenic cell, tissue or organism according to the invention as described herein. The method preferably  
25           comprises contacting the compound with a transgenic cell, tissue or organism according to the invention as described above, and screening for a phenotypic change in the cell, tissue or organism. Preferably the compound comprises an inhibitor or enhancer of a protein of the signal transduction pathway of the  
30           cell, tissue or organism of which UNC-53 is a component or is an inhibitor or enhancer of a parallel or redundant signal transduction pathway. Such enhancers or inhibitors are defined by particular phenotypic changes in the transgenic cell, tissue or  
35           organism, for example changes in cell shape or mobility or the direction of cell migration.



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Preferably the compound is an inhibitor or an enhancer of the activity of UNC-53 protein of C. elegans or a functional equivalent, derivative or bioprecursor thereof, which protein is expressed in the transgenic cell, tissue or organism as defined herein.

Preferably the phenotypic change to be screened comprises a change in cell shape or a change in cell motility. Where a transgenic cell is used in accordance with one embodiment of the method of the invention, an N4 neuroblastoma cell may be used and in such an embodiment the phenotypic change to be screened may be the length of neurite growth or changes in filipodia outgrowth or alternatively changes in ruffling behaviour or cell adhesion. In an alternative embodiment of the method of the invention, the transgenic cell may comprise an MCF-7 breast carcinoma cell. Typically in such an embodiment the phenotypic change to be screened comprises the extent of phagokinesis. The method according to the invention, may also utilise a mutant cell or mutant organism according to the invention as described above, where the mutant cell is capable of growing in tissue culture and either of which cell or organism has a mutation in the wild-type unc-53 gene.

In accordance with the present invention, a "phenotypic change", may be any phenotype resulting from changes at any suitable point in the life cycle of the cell, tissue or organism defined above, which change can be attributed to the expression of the transgene such as for example, growth, viability, morphology, behaviour, movement, cell migration or cell process or growth cone extension of cells and includes changes in body shape, locomotion, chemotaxis, mating behaviour or the like. The phenotypic change may preferably be monitored directly by visual inspection or alternatively by for example

measuring indicators of viability including endogenous or transgenically introduced histochemical markers or other reporter genes, such as for example  $\beta$ -galactosidase.

5           A compound which is identifiable by the method according to the invention as described above, as an enhancer of the regulation of cell shape or motility or the direction of cell migration in C. elegans may be used as a medicament, or alternatively in the  
10       preparation of a medicament, for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries. Examples of promoting neuronal regeneration include for example peripheral  
15       nerve regeneration after trauma and spinal cord trauma.

          Where a compound is identified in accordance with the method described above as being an inhibitor of the regulation of cell shape, the compound may be used  
20       as a medicament, or in the preparation of a medicament, for substantially alleviating spread of disease inducing cells, such as in spread of cancers, or the like in metastasis. Advantageously, any of the compounds which may have been identified as an  
25       inhibitor or an enhancer in accordance with the method as described above, may also be included in a pharmaceutically acceptable formulation comprising the respective compound and an acceptable carrier, diluent or excipient therefor.

30           The particular mechanism of action of a compound identified as either an inhibitor or an enhancer of the cell motility or direction of cell migration is not limiting preferably the compound acts as an inhibitor or enhancer of a signal transduction pathway  
35       downstream. The compound may also act on parallel pathway or on the UNC-53 protein of C. elegans. For

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example, the method of action of the compound may include direct interaction with UNC-53 protein, interaction with processes for regulating phosphorylation of UNC-53 or for processes regulating activity of an unc-53 gene or for processes for post-transcriptional or post-translational modification or the like.

Preferably the compound is identified by the method according to the invention as an inhibitor or an enhancer, by utilising differences of phenotype of the cell, tissue or organism, which are visible to the eye. Alternatively indicators of viability including endogenous or transgenically introduced histochemical markers or a reporter gene may be used.

According to a further aspect of the invention there is also provided a transgenic cell or tissue culture which has been constructed to comprise a promoter sequence of an unc-53 gene of C. elegans or a functional fragment thereof, fused to a nucleic acid sequence encoding a reporter molecule. Preferably, the reporter sequence encoding the reporter molecule encodes for a detectable protein, for example one which may be monitored by eye inspection such as antibiotic resistance,  $\beta$ -galactosidase or a molecule detectable by spectrophotometric, spectrofluorometric, luminescent or radioactive assays. Preferably the reporter molecule is green fluorescent protein (GFP), which advantageously allows inhibition or enhancement of the UNC-53 protein according to the invention to be monitored visually.

The present invention also provides a method of determining whether a compound is an inhibitor or an enhancer of transcription of a an unc-53 gene in C. elegans, or a functional fragment thereof, which method comprises the steps of:

(a) contacting said compound with a transgenic

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cell according to the further aspect of the invention as described above,

(b) monitoring the reporter molecule and comparing results obtained from this monitoring step with a control comprising a transgenic cell having the promoter sequence of an unc-53 gene, or a functional fragment thereof and the reporter molecule, in the absence of the compound.

In one embodiment of the method according to the invention the reporter molecule may comprise messenger RNA. Alternatively the reporter molecule may be green fluorescent protein (GFP).

A compound identified as an inhibitor or enhancer of transcription of the unc-53 gene or a fragment thereof may also be used as a medicament, or in the preparation of a medicament, for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries. Furthermore, such compounds may be included in a pharmaceutical formulation including a carrier, diluent or excipient therefor.

The present invention also provides a kit for determining whether a compound is an enhancer or an inhibitor of the regulation of cell motility or shape or the direction of cell migration, which kit comprises at least a plurality of transgenic or mutant cells according to the invention as described above and a plurality of wild-type cells of the same cell type or cell line or tissue culture.

Also provided by the present invention is a kit for determining whether a compound is an inhibitor or an enhancer of transcription of an unc-53 gene of C. elegans or a functional fragment thereof, which comprises at least a plurality of transgenic cells as described above and means for monitoring the reporter

molecule.

For the purposes of the present invention, the term "unc-53 gene or a functional fragment thereof" includes the nucleic acid sequence shown in Figure 1 or a fragment thereof, including the differentially spliced isoforms and transcriptional start of the unc-53 gene sequence and which sequence encodes an UNC-53 protein or a functional equivalent, derivative, fragment or bioprecursor of the protein.

The present invention also provides an oligonucleotide probe which comprises the carboxy-terminal 1.5 kb of the coding nucleic acid sequence shown in Figure 1 or a fragment thereof comprising not less than 15 base pairs. In addition, the present invention provides a further oligonucleotide probe comprising a nucleic acid sequence encoding the amino acid sequence as numbered 1 to 10 and 14 to 133, 487 to 495, 537 to 545, 1032 to 1037, 1097 to 1116 or 1300 to 1307, as shown in Figure 3 or a fragment thereof comprising between 18 and 24 base pairs. The oligonucleotide probes described above may also be advantageously be labelled for detection.

The present invention also provides methods of identifying C. elegans genes or fragments thereof, which encode proteins which are active in the signal transduction pathway of which UNC-53 is a component and which are homologues of UNC-53. A preferred method comprises hybridizing to a C. elegans cDNA library an oligonucleotide probe according to the invention as described above, under appropriate conditions or stringency in order to identify genes having statistically significant homology with the cDNA clones of any one of the cDNA sequences according to the invention described above.

Furthermore, there is also provided by the present invention a method of identifying a protein

which is active in the signal transduction pathway of a cell. According to this aspect of the invention, the method comprises;

- 5 (a) contacting an extract of said cell with an antibody to the UNC-53 protein or a functional equivalent, fragment or bioprecursor thereof,
- (b) identifying the antibody/UNC-53 complex, and
- (c) analysing the complex to identify any protein bound to the UNC-53 protein other than  
10 the antibody.

The UNC-53 protein, therefore may bind regions of other proteins involved in the signal transduction pathway. It is also possible to sequentially identify a whole range of proteins involved in the signal  
15 transduction pathway. This aspect of the invention, further comprises a method of identifying a further protein or proteins which are active in the signal transduction pathway of a cell which method comprises:

- 20 (a) forming an antibody to the identified protein bound to the UNC-53 protein in the method as described above,
- (b) contacting a cell extract of C. elegans with the antibody,
- (c) identifying the antibody/protein complex,
- 25 (d) analysing the complex to identify any further protein bound to the first protein other than the antibody, and
- (e) optionally repeating steps (a) to (d) to identify further proteins in the pathway.

30 According to this aspect of the present invention, the antibody, which is preferably a monoclonal antibody, such as for example monoclonal antibody designated as 16-48-2, starts the process by binding to the UNC-53 protein or a functional  
35 equivalent thereof in the signal transduction pathway. Any other proteins found complexed to the bound

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antibody or UNC-53 protein can then be used to identify further interacting proteins involved in the pathway.

5 It may also be possible to identify proteins involved in the signal transduction of a cell by using UNC-53 protein of C. elegans. According to this aspect of the invention the method comprises:

- 10 (a) contacting an extract of the cell with the UNC-53 protein of C. elegans or a functional equivalent, fragment or bioprecursor of said UNC-53 protein
- (b) identifying the UNC-53 protein/protein complex and
- 15 (c) analysing the complex to identify any protein bound to the UNC-53 protein other than another UNC-53 protein

20 This method can also advantageously be used to identify further proteins in a signal transduction pathway of a cell by contacting an extract of the cell used as described above, with any protein identified from step (c) above not being an UNC-53 protein and  
25 repeating steps (b) and (c).

Other methods which may be used for identifying proteins in a signal transduction pathway of a cell may comprise for example a western blot overlay method  
30 which method is well known to those skilled in the art. Cell extracts are run on SDS-gels to separate out protein and subsequently blotted onto a nylon membrane. These membranes may then be incubated, for example in a medium containing UNC-53 with a biotin  
35 label thereon and any protein conjugates visualised

with a streptavidin-alkaline phosphatase conjugated antibody.

5       The present invention also advantageously provides a process for the preparation of binding antibodies which recognise proteins or fragments thereof involved in the rate and direction of cell migration or the control of cell shape, for the above methods. Preferably the antibody is monoclonal  
10       antibody and more preferably monoclonal antibody 16-48-2.

      The monoclonal antibody for binding to UNC-53 (or its functional equivalent) may be prepared by known techniques as described by Kohler R. and Milstein C.,  
15       (1975) Nature 256, 495 to 497.

      Another method which may be used to identify proteins involved in the signal transduction pathway involves investigating protein-protein interactions using the two-hybrid vector method. This method,  
20       which is well known to those skilled in the art, utilises the properties of the GAL4 protein in yeast. GAL4 is a transcriptional activator of galactose metabolism in yeast and has a separate domain for binding to activators upstream of the galactose  
25       metabolising genes as well as a protein binding domain. Nucleotide vectors may be constructed, one of which comprises the nucleotide residues encoding the DNA binding domain of GAL4. These binding domain residues may be fused to a known protein encoding  
30       sequence, such as for example unc-53. The other vector comprises the residues encoding the protein binding domain of GAL4. These residues are fused to residues encoding a test protein, preferably from the signal transduction pathway of C. elegans. Any  
35       interaction between the UNC-53 protein and the protein to be tested leads to transcriptional activation of a



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reporter molecule in a GAL-4 transcription deficient yeast cell into which the vectors have been transformed. Preferably, a reporter molecule such as  $\beta$ -galactosidase is activated upon restoration of transcription of the yeast galactose metabolism genes. This method enables any interactions between proteins involved in the signal transduction pathway to be investigated.

Any proteins identified in the signal transduction pathway of the cell, which may be for example a mammalian cell, may also be included in a pharmaceutical composition together with a carrier, diluent or excipient therefor.

The present invention also provides a process for producing an UNC-53 protein of C. elegans or a functional equivalent, fragment, or derivative of the protein, which process comprises culturing the cells transformed or transfected with a cDNA expression vector having any of the cDNA sequences according to the invention as described above, and recovering the expressed UNC-53 protein. The cell may advantageously be a bacterial, animal, insect or plant cell.

A particularly preferred process for producing UNC-53 protein comprises using insect cells. Accordingly, the invention provides a process for producing an UNC-53 protein of C. elegans or a functional equivalent, fragment, derivative or bioprecursor of the UNC-53 protein, which process comprises culturing an insect cell transfected with a recombinant Baculovirus vector, said vector comprising a nucleotide vector encoding the UNC-53 protein or a functional equivalent, fragment or bioprecursor thereof downstream of the Baculovirus polyhedrin promoter and recovering the expressed UNC-53 protein. Advantageously, this method produces large amounts of protein for recovery. The insect cell may be from for

example Spodoptera frugiperda or Drosophila  
Melanogaster.

In accordance with the present invention, a defined nucleic acid sequence includes not only the  
5 identical nucleic acid but also any minor base variations from the natural nucleic acid sequence including in particular, substitutions in bases which result in a synonymous codon (a different codon specifying the same amino acid), due to the degenerate  
10 code in conservative amino acid substitution. The term "nucleic acid sequence" also includes the complimentary sequence to any single stranded sequence given which includes the definition above regarding base variations.

15 Furthermore, a defined protein, polypeptide or amino acid sequence according to the invention, includes not only the identical amino acid sequence but also minor amino acid variations from the natural amino acid sequence including conservative amino acid  
20 replacements (a replacement by an amino acid that is related in its side chains). Also included are amino acid sequences which vary from the natural amino acid but result in a polypeptide which is immunologically identical or similar to the polypeptide encoded by the  
25 naturally occurring sequence. Such polypeptides may be encoded by a corresponding nucleic acid sequence.

The invention may be more clearly understood from the following description with reference to the accompanying drawings and photographs, in which

30 Fig. 1 shows one strand of the C. elegans unc-53 mRNA translated into DNA (U to T) (5073 bases) which corresponds to the 8A clone variant encoding the corresponding 8A protein shown in Figure 3. Designations "TB" are positions onto which SL1  
35 transplacers have been identified at the 5' end of the sequence. Different mRNAs which differ in their 5'

end therefore exist. Potential start methionines are double underlined (M). Restriction endonuclease sites are indicated. A region of 8 sequential A bases at positions 4594 to 4601 is underlined. This region  
5 differs from the corresponding region of the known sequence in the database (F45E10.1) by having 8 rather than 7 A'denine (A) bases resulting in a frame shift (see Fig 15) and corresponds to the 7A form of the protein. The nucleic acid sequence from the database  
10 is also included in the nucleic acid sequences of the present application for reference only.

Fig. 2 shows a comparison of the sequences of the 7A and 8A clones of Figure 1.

Fig. 3 shows the predicted C. elegans amino acid  
15 UNC-53 sequence corresponding to the nucleic acid sequence of the 8A clone shown numbered from 1 to 1528. Again, potential start methionines are double underlined (M). Designations "tb" are regions for PCR clones to identify PCR products. Other regions of  
20 interest are identified. The region indicated as S4 is part of a lambda clone - 16.8 kb of the UNC-53 nucleic acid. This sequence, when translated is part only of the UNC-53 protein. Yet, injection of this part gives transformation rescue in organisms, i.e.  
25 providing additional evidence for the existence of shorter forms of the protein.

Fig. 4 shows the predicted C. elegans amino acid sequences of Figure 3 in the three letter code for indicating amino acids.

30 Fig. 5 shows the predicted C. elegans amino acid sequence UNC-53 sequence corresponding to the nucleic acid sequence of the 7A clone of Figure 2 shown numbered from 1 to 1583.

Fig. 6 shows the amino acid sequence of Figure 5  
35 in the corresponding three letter code format for indicating amino acids.

Fig. 7 shows sequences of low complexity of the amino acid sequence of the corresponding nucleic acid sequence of the 8A clone of Fig. 3 identified with the filter and SEG algorithms of the BLAST sequence  
5      homology package. Regions of low complexity are indicated by "X" for the first copy of the sequence and by underlined amino acids for the second copy.

Fig. 8 shows, schematically, the known branches  
10      of the highly conserved Receptor Tyrosine Kinase/GRB2 signal transduction pathway including UNC-53.

Fig. 9 shows, schematically, the differences in cells with increased and decreased UNC-53 expression from the wild type.

Fig. 10 is a graph of the effect of anterior-  
15      posterior signal strength on growth cone extension rate of C. elegans organisms, with increased and decreased UNC-53 expression from the wild type. This graph translates the observation that UNC-53 acts in a dosage-dependent way to direct the rate of extension  
20      in the anterior/posterior axis into a model. The signal received e.g. (egl-15) is an RTK mediated signal which is postulated to be received by UNC-53 and which results in extension in the anterior/posterior axis. The graph shows an allelic  
25      series of organisms with a graded reduction in extension from increased UNC-53 expression down through wild type to a reduced UNC-53 expression. The prediction is thus: for the same level of RTK mediated signal the increased/decreased growth in the  
30      anterior/posterior axis depends on the level of expression of UNC-53 in any organism. The graph also reflects the prediction that for organisms with a particular level of UNC-53 overexpression there is no requirement for a signal before growth cone extension  
35      occurs. This extension is likely to be in a random direction or influenced by alternative factors.

Fig. 11 shows constructs of unc-53 nucleic acid including identified functional domains .

Fig. 12 shows 5' amino terminus of the cDNA encoding from the first methionine amino acid through the actin binding protein homology domain (amino acids 1-133 from Fig. 1) and oligonucleotides designated oligo BG01, BG02 and BG03 (amplification strategies of amino terminus of the unc-53 cDNA). Combinations of oligo BG02 with either oligo BG02 or BG03 were used to amplify the 5' terminus of the cDNA from the first methionine through the actin binding protein homology domain (amino acids 1-133). All of the oligonucleotides are underlined and sequences identical to the cDNA are shown in upper-case. In addition to unc-53 sequence, oligo BG02 contains a stop codon and the recognition sequence for BamHI endonuclease. Oligo BG01 has engineered EcoRI and NdeI recognition sites for inclusion in bacterial expression vectors. Both constructs remove the 5' untranslated region of unc-53 and oligo BG03 contains a NotI cleavage site. Oligo BG03 has an improved ribosome binding site similar to mammalian ribosome binding sites. Use of BG03 in PCR thus results in constructs optimised for mammalian expression.

Figure 13 shows, schematically, constructs of the plasmids pTB109, pTB110, pTB111 and pTB112.

Fig. 14(a) shows a summary of transcript starts at the 5' end of the unc-53 gene. Different identified transcript starts and corresponding in-frame ATG-codons are marked. Tab2 is the oligo from within cDNA M5 which was used in RT PCR experiment to identify/isolate the 5' ends of different UNC-53 mRNAs.

Figure 14(b) shows the location of the different transcript starts on the genomic DNA and the position of the S4 Lambda clone with respect to genomic DNA.

Figure 14(c) shows the sequence near the 5' and 3' ends of the lambda S4 clone, identifying its composition corresponding to the 5' and at position 2260 of comid COGH10 and the 3' end of F45R10 at position 3287.

Fig. 15 shows the alignment of UNC-53 protein with the carboxytermini of the  $\alpha$ -actinin and  $\beta$ -spectrin family (QY is UNC-53).

Fig 16 shows the predicted actin binding sites of UNC-53. The comparison shows internal LKK repeats.

Fig. 17 shows the alignment of the candidate SH3 binding sites in UNC-53 with known SH3 sites of other named proteins. Proteins at positions 4 and 7 are critical for binding into SH3 pockets.

Fig. 18 shows the alignment of the predicted amino acid sequences from F45E10.1 (available in public database) with UNC-53. The different identified amino acid is shown at position 1186. The frameshift which results in the different amino acid sequence from position 1513 is a result of the different number of adenine bases in the nucleic acid sequence (see Fig. 1).

Fig. 19 is a series of photographs of C. elegans embryos (strain TB4Ex25 (Table 1) [UNC-53-UNC-54 construct]). The photographs show increased outgrowth in the anterior-posterior axis of body wall cells in the C. elegans embryos which overexpress UNC-53 (immunofluorescence with UNC-53 mab 16-48-2) Individual photographs are as follows:

- A: early embryo comma stage
- B: 1.5 fold stage embryo
- C: 3 fold stage embryo, first plane of focus
- D: 3 fold stage embryo, second plane of focus
- E: 3 fold stage, mosaic animal, 3-cells in a quadrant giving expression.

This demonstrates that immunofluorescence

provides a measure of the expression in the transgenic lines of UNC-53.

Fig. 20A is a photograph of C. elegans embryo containing DNA construct pTB110 (strain TBAIn76(table 1)). Shown is expression of UNC-53 following heat shock.

Fig. 20B and C are photographs of C. elegans embryos containing DNA construct pTB111 (strain TB1Ex6 (table 1)). Shown is transgenic expression of UNC-53 in mechano-sensory neurons.

Fig. 21 shows photographs of the following:

- A: A wild-type UNC-53 L1 larva of genotype 4-25 (strain TB4Ex25) as in photographs 19B, C and D.
- B: L1 larva of 4-25 with morphological defects associated with muscle abnormalities.
- C: Lethal phenotype of 4-25.
- D: L1 larva of 4-25 showing misshapen animal and muscle cells with increased extensions. Also shows constipation problems associated with abnormal muscle pattern.
- E: L1 larva of the heat-shock line TBAIn76 (table 1) exhibiting morphological abnormalities following heat shock and recovery.
- F: L1 larva of line TBAIn76 (table 1) showing morphological defects in the pharynx.

All Figs. 19, 20 and 21 are Normarski optics of live embryos.

Fig. 22 is a map of plasmid pTB110 (tables 1 and 2) a heat shock promoter fusion, indicating restriction endonuclease sites.

Fig. 23 is a map of plasmid pTB112 (tables 1 and 2) a muscle specific UNC-54 fusion, indicating restriction endonuclease sites.

Fig. 24 is a map of plasmid pTB54 (the 8A clone variant) (tables 1 and 2). In the construction of this plasmid the complete unc-53 cDNA (tb3M5) of the

8A variant, including 5' and 3' UTRs was cloned as a NotI-ApaI fragment into the mammalian expression vector pcDNA3 (Invitrogen).

5 Figure 25 is a map of plasmid pTB72 (the construct encoding the 7A clone variant of UNC-53 cDNA of Figure 2).

Figure 26 is nucleotide sequence of the plasmid map of Figure 25.

Figure 27 is a map of plasmid pTB73.

10 Figure 28 is a nucleotide sequence of plasmid pTB73 of Figure 27.

Figure 29 is a map of plasmid pCB50.

Figure 30 is a nucleotide sequence of plasmid pCB50 of Figure 29.

15 Figure 31 is a map of plasmid pCB51.

Figure 32 is a nucleotide sequence of the plasmid pCB51 of Figure 31.

Figure 33 is a map of plasmid ppCB55.

20 Figure 34 is a nucleotide sequence of plasmid pCB55 of Figure 33.

Figure 35A illustrates a flowchart of the actin co-sedimentation assay. Soluble UNC53 protein was incubated with monomeric G-actin in a buffer containing ATP. Polymerization of G-actin to F-actin was induced by increasing the salt concentration to 100 mM, F-actin protein complexes were collected by centrifugation and analyzed by SDS-PAGE and fluorography.

30 Figure 35(B) illustrates the concentration series of the actin co-sedimentation assay. The full length UNC-53 encoding cDNA (pTB72) was transcribed and translated in vitro and co-sedimented with F-actin at a starting G-actin concentrations ranging from 0 to 250 mg/ml. See methods for details. S=supernatant after airfuging. P=pellet after airfuging.

35 Figure 35(C) illustrates both the full length



(pTB72) and amino terminal deleted UNC53 (pTB73) protein co-sediment with F-actin. Starting G-actin concentration was 500 mg/ml. S=supernatant, P=pellet, R= starting *in vitro* reaction.

5        Figure 36(A) is a flowchart of a SEM-5 binding experiment. The truncated UNC53 cDNA (pTB50) was transcribed and translated *in vitro* and incubated with SEM5-GST sepharose or GST sepharose. After four  
10        washes, the remaining proteins bound to the matrix were analyzed by SDS-PAGE and fluorography.

      Figure 36(B) illustrates an immunoprecipitation experiment of radioactively labelled UNC53 proteins from the TnT pTB50 reaction shows that monoclonal antibody 16-48-2 recognizes both the native (-SDS  
15        lanes) and denatured (+SDS) protein products *in vitro*. c=control reaction without anti-UNC53 monoclonal antibody 16-48-2. ab=reaction with monoclonal antibody 16-48-2. See methods for details.

      Figure 36(C) illustrates the results of SEM-5-GST  
20        binding experiments outlined in (a). *In vitro* translated UNC53 protein were analyzed by SDS-PAGE and fluorography. See methods for details.  
      sup=supernatant

      Figure 36(D) illustrates a western blot overlay  
25        experiment of UNC-53 (construct pTB61) expressed in bacterial cells. Cell lysates were denatured in Laemmli buffer and the proteins separated by 5-25% gradient SDS-PAGE. The arrowhead indicates the presence of full length UNC-53 in the induced  
30        bacterial lysate. Additional gels were blotted to nylon membrane, incubated with biotinylated GST or biotinylated GST-GRB2 protein and bound protein complexes subsequently detected with a streptavidin-alkaline phosphatase conjugated antibody. See methods  
35        for details. U=uninduced bacterial cell lysate, I=induced bacterial cell lysate.

Figure 37 is a series of photographs of C. elegans which illustrates overexpression of UNC-53 in body muscle cells results in over-extension along the longitudinal axis. Transgenic C. elegans embryos carrying the construct pTB113 were analyzed for UNC-53 activity by immunohistochemistry with the 16-48-2 antibody. Starting from the photograph (a) of the top left panel of Figure 37.

(A) and (B) illustrate ectopic growth cone spikes (indicated by the arrowheads) are observed early in myogenesis in the comma stage embryo. (C) and (D) illustrate over-extension of muscle cells in the head region of a three fold embryo during outgrowth. (E) illustrates over-extension is clearly observed along the anterior-posterior axis (indicated by the arrowheads) of a late 3 fold embryo.

Figure 38 is a map of plasmid ptb113.

Figure 39 is a nucleotide sequence of the plasmid ptb113 of Figure 38.

Figure 40 illustrates neurite tree length and fraction positive cells enhancement in a transfected cell C9 compared to wild-type cells C0. Black bars indicate fraction positive cells whereas hatched bars indicate neurite tree length cells, as described in example 8.

Figure 41 illustrates the results obtained following application of compound (I-(IH-pyrrol-2-ylmethyl)-2-piperidinone) to N4 transfected cells. The dark coloured bars indicate fraction positive C0 clones whereas the hatched bars of the chart indicate fraction positive C9 clones.

The following sequence listings are referred to in the specification.

Sequence 1D No 1: is a nucleic acid sequence

corresponding to the 7A nucleic acid sequence variant of Figure 2.

5 Sequence 1D No 2: is a nucleic acid sequence corresponding to the 8A nucleic acid sequence variant of figure 1.

10 Sequence 1D No 3: is an amino acid sequence corresponding to the amino acid sequence of the 8A variant of figure 3.

15 Sequence 1D No 4: is an amino acid sequence corresponding to the amino acid sequence of the 7A variant of figure 2.

Sequence 1D No 5: is an amino acid corresponding to the amino acid sequence shown in figure 7.

20 Sequence 1D No 6: is a nucleic acid sequence of the oligo BGO3 sequence of figure 12.

Sequence 1D No 7: nucleic acid sequence of the oligo BGO1 sequence of figure 12.

25 Sequence 1D No 8: is a nucleic acid sequence of the oligo BG02 sequence of figure 12.

30 Sequence 1D No 9: is an amino acid sequence corresponding to the amino acid UNC-53(a) sequence shown in figure 17.

35 Sequence ID No 10: is an amino acid sequence corresponding to amino acid sequence of sequence (b) of UNC-53 shown in figure 17.

Sequence ID No 11: is an amino acid sequence

corresponding to the sequence (c) of an SOS shown in figure 17.

5 Sequence ID No 12: is an amino acid sequence corresponding to the sequence (d) of an SOS shown in figure 17.

10 Sequence ID No 13: is an amino acid sequence corresponding to the sequence (d) of an SOS shown in figure 17.

15 Sequence ID No 14: is an amino acid sequence corresponding to the sequence (f) of SOS 1359 shown in figure 17.

Sequence ID No 15: is an amino acid sequence corresponding to the sequence (g) of SOS 1377 shown in figure 17.

20 Sequence ID No 16: is an amino acid sequence corresponding to the sequence (h) of Dynamin shown in figure 17.

25 Sequence ID No 17: is an amino acid sequence corresponding to the sequence (i) of dynamin shown in figure 17.

30 Sequence ID No 18: is an amino acid sequence corresponding to the sequence (j) of PI3K p85 shown in figure 17.

35 Sequence ID No 19: is an amino acid sequence corresponding to the sequence (k) of P13k p85 shown in figure 17.

Sequence ID NO 20: is an amino acid sequence

corresponding to the sequence (l) of AFAP-110 shown in figure 17.

5 Sequence No 21: is an amino acid sequence  
corresponding to the sequence (m) of AFAP-110 shown in figure 17.

10 Sequence No 22: is an amino acid sequence  
corresponding to the sequence (n) of 3BP-1 shown in figure 17.

15 Sequence ID No 23: is an amino acid sequence  
corresponding to the sequence (o) of 3BP-1 shown in figure 17.

Sequence ID No 24: is an amino acid sequence which corresponds to the amino acid sequence from positions 106 to 133 of UNC-53 shown in figure 16.

20 Sequence ID No 25: is an amino acid sequence which corresponds to the amino acid sequence from positions 1093 to 1120 of UNC-53 shown in figure 16.

25 Sequence ID No 26: is a nucleotide sequence  
corresponding to the nucleotide sequence of ptB72 shown in figure 26.

30 Sequence ID No 27: is a nucleotide sequence  
corresponding to the nucleotide sequence of ptB73 shown in figure 28.

35 Sequence ID No 28: is a nucleotide sequence  
corresponding to the nucleotide sequence of pCB50 shown in figure 30.

Sequence ID No 29: is a nucleotide sequence

corresponding to the nucleotide sequence of pCB51  
shown in figure 32.

5      Sequence ID No 30: is a nucleotide sequence  
corresponding to the sequence of pCB55 shown in figure  
34.

10      Sequence ID No 31: is a nucleotide sequence  
corresponding to the nucleotide sequence of ptb113  
shown in figure 39.

15      Sequence ID No 32: is an amino acid sequence  
corresponding to the amino acid sequence as numbered  
from amino acid 1 to 110 of the sequence figure 3.

Sequence ID No 33: is an amino acid sequence  
corresponding to the sequence as numbered from amino  
acid sequence 114 to 133 of the sequence of figure 3.

20      Sequence ID No 34: is an amino acid sequence  
corresponding to the sequence as numbered from amino  
acid sequence 487 to 495 of the sequence of figure 3.

25      Sequence ID No 35: is an amino acid sequence  
corresponding to the sequence as numbered from amino  
acid sequence 537 to 545 of the sequence of figure 3.

30      Sequence ID No 36: is an amino acid sequence  
corresponding to the sequence as numbered from amino  
acid sequence 1032 to 1037 of the sequence of figure  
3.

35      Sequence ID No 37: is an amino acid sequence  
corresponding to the sequence as numbered from amino  
acid sequence 1097 to 1116 of the sequence of figure  
3.

Sequence ID No 38: is an amino acid sequence corresponding to the sequence as numbered from amino acid sequence 1300 to 1307 of the sequence shown in figure 3.

5

Sequence ID No 39: is an amino acid sequence corresponding to the amino acid sequence (a) of  $\alpha$ -actinin (aact) shown in figure 15.

10 Sequence ID No 40: is an amino acid sequence corresponding to the amino acid sequence (b) of unc-53 shown in figure 15.

15 Sequence ID No 41: is an amino acid sequence corresponding to the amino acid sequence (c) of  $\beta$ -spectrin (spectrin) shown in figure 15.

20 Sequence ID No 42: is an amino acid sequence corresponding to the amino acid sequence (d) of  $\alpha$ -actinin (aact) shown in figure 15.

Sequence ID No 43: is an amino acid sequence corresponding to the amino acid sequence (e) of UNC-53 shown in figure 15.

25

Sequence ID No 44: is a amino acid sequence corresponding to the amino acid sequence (f) of  $\beta$ -spectrin (spectrin) shown in figure 15.

30 Sequence ID No 45: is an amino acid sequence corresponding to the amino acid sequence (g) of  $\alpha$ -actinin shown in figure 15.

35 Sequence ID No 46: is an amino acid sequence corresponding to the amino acid sequence (h) of UNC-53 shown in figure 15.

Sequence ID No 47: is an amino acid sequence corresponding to the amino acid sequence (I) of  $\beta$ -spectrin shown in figure 15.

- 5      Sequence ID No 48: is a nucleotide sequence corresponding to the nucleotide sequence of S4 lambda clone shown in figure 14(c).

10            The inventors have established a set of processes particularly in C. elegans to select for inhibitors or enhancers of UNC-53. This screen is based on transgenic or mutant organisms or cells in which we have introduced a nucleic acid sequence encoding UNC-  
15      53 under the control of a specific promoter. In these organisms UNC-53 is over-stimulated as judged by increased extension of growth cones of muscle cells which over-express UNC-53 in C. elegans. This leads to a range of phenotypes in both embryonic and  
20      postembryonic development (from death to defective morphology and motility). These phenotypes can be scored with simple means at high throughput. Similar results can be obtained with heat shock specific lines. The basis of our test for inhibitors of the  
25      UNC-53 signal transduction pathway is reversal of this phenotype to an improved state of health.

          We have constructed transgenic strains of C. elegans which over-express UNC-53 in body muscle. This results in increased extension of muscle cells  
30      and embryonic lethality (17 to 80% of transgenic organisms depending on the line used). These strains are used to directly screen for drugs which interfere with unc-53 genes, UNC-53 protein activity or any regulatory factor thereof to thereby suppress the  
35      background lethality.

          Another process which may be used for selecting



inhibitors or enhancers of UNC-53 uses a constitutively active unc-53. This is achieved by mutating the nucleotide binding domain such that GTP or ATP is always bound or by covalently attaching SEM-5. In this strategy, transgenics (tissue cultured cell lines, or organisms such as nematodes) are generated which maintain unc-53 in a higher endogenous level of activity. Over-extension and subsequent lethality results in a greater proportion than that observed in the UNC-54/UNC-53 wild type lines. By screening for survivors after drug treatment, this assay specifically identifies inhibitors of downstream components in the signal transduction pathway.

Another process utilises an UNC-53 promoter. In this approach, an UNC-53 promoter is fused to a nucleic acid sequence encoding a reporter molecule, for example green fluorescent protein (GFP). Cells will glow when trans-acting factors bind to the promoter to activate transcription. By screening for cells which do not fluoresce, molecules which inhibit transcription of UNC-53 are identified.

The processes for selecting inhibitors and/or enhancers according to the invention are preferably carried out on whole animals. This can be done using a C. elegans system. The advantages of these tests include:

- (1) The screening in a whole animal assay. C. elegans is a complex multicellular organism with a full nervous system, digestive system, etc. Its anatomy and development has been described in extreme detail. It is one of the best-characterised higher organisms at the genetic, molecular, developmental and cell biological level. Any observed changes to phenotype can be checked against this database.
- (2) To study effects on rate and directionality of cell migration and the change of cell shape it is

important to leave the cells under study in a setting where they are surrounded by the in vivo interacting tissues, cells and substrates for cell migration etc. This can be done using whole C. elegans subjects. A situation has been created where the given pathway is over-stimulated leading to an easily scorable phenotype which can be reverted in any assay or process.

(3) The endpoint of the screen is the substantially increased health of the organism. This permits the exclusion of non-specific and toxic compounds.

(4) A complete and specific inhibition of UNC-53 in the transgenics will lead at the worst to the phenotype of an UNC-53 reduction or loss of function mutant which we have described, can recognise and have shown not to be essential for viability.

(5) The test can be adapted to make full use of the advantages of the C. elegans model system such as the possibility to conduct the test chronically over several generations and the possibility to conduct the test in different genetic backgrounds, e.g. RTK constitutive or defective.

(6) C. elegans exhibits a complex set of wild type, drug- and mutation-induced phenotypes such as changes in body shape, subtle changes in locomotion, mating behaviour, chemotaxis, pharyngeal pumping, egg laying behaviour, which can be used as part of a phenotype analysis or screen.

The results of C. elegans research described herein has provided important breakthroughs in biomedical research fields such as programmed cell death, neuronal guidance, the Receptor Tyrosine Kinase/RAS signal transduction pathway, integrin/cell adhesion receptor signalling, etc.,

The biochemical association of UNC-53 in the RTK signal transduction pathway enables identification of

genes or of biochemical pathways which are targets for pharmacologically or pharmaceutically active compounds and the development of high throughput and mode of action specific drug screens using wild type, mutant and transgenic animal strains including, in particular, C. elegans.

Thus pharmacological manipulation of the UNC-53 pathway is now possible on the following rationale:

We have scientific arguments to expect C. elegans UNC-53 to interact in vivo with the other components of RTK signal transduction pathways based on:

(1) The observation that C. elegans SEM-5 and GRB-2 are mutually exchangeable in vivo, combined with our observed in vitro binding of both GRB-2 and SEM-5 to UNC-53. Thus, C. elegans UNC-53 will be able to interact with the activated GRB-2/RTK receptor in mammalian cells.

(2) UNC-53 interacts with the rabbit actin-cytoskeleton

Expression of C. elegans UNC-53 in mammalian cell lines represents a shortcut to develop pharmacological assays and screens to target this pathway. We have shown that over-expression of the C. elegans UNC-53 in C. elegans myoblasts leads to over-extension of these cells in the anterior/posterior axis of the embryo and ultimate disorganisation of the muscle cell and myofilament pattern. (Over)-expression of C. elegans UNC-53 in a human cell line leads to a detectable change in phenotype, in particular increased motility of cells, increased outgrowth of neurons and morphological changes in the elongation and cytoskeletal morphology of differentiating myotubes.

The C. elegans unc-53 Open Reading Frame (ORF) (with and without optimised Kozak consensus sequence) of both 7A and 8A clone variants has been cloned between the CMV major intermediate early

promoter/enhancer and bovine growth hormone polyA  
signal sequence of expression vector pcDNA3  
(Invitrogen). This vector is designed for high level  
stable and transient expression in most mammalian  
5 cells.

The following additional considerations require  
mention:

(1) Genetic analysis of reduction in UNC-53 function  
and ectopic expression experiments suggest that UNC-53  
10 acts in a highly dosage-dependent manner. As is the  
case for RAS, increased expression may lead to  
lowering the threshold of RTK-signal required for a  
given response or may remove the requirement for an  
activating signal to obtain a phenotype response (Fig  
15 10). In addition UNC-53 is an unusually low abundance  
protein in wild type C. elegans. It is therefore  
likely to be necessary or useful to control the  
temporal and quantitative expression of UNC-53 in the  
proposed assay conditions in all organisms or cells to  
20 be assayed. The already available or a further  
optimised expression cassette is then cloned in  
expression vectors with IPTG- inducible or  
tetracycline-repressible promoters. It is realised  
that both the Lac and Tet expression systems are  
25 leaky. Additional other repressible/inducible  
expression systems (e.g. Mx promoter) or weak  
mammalian promoters might be preferred.

(2) Over-expression of the endocytosis controlling  
protein dynamin leads to phenotypes which are not  
30 associated with dynamin function in the cell but which  
are thought to be due to sequestration of the GRB-2  
pool in the cell (GRB-2 is an adaptor for a variety of  
signal transduction pathways). Such sequestration is  
unlikely to lead to "positive effects" on the activity  
35 of the cell such as is observed in the presently  
described assay system (increased cell process

extension or motility), see Fig 19. Based on the  
homology between UNC-53 and GTP-binding, we can also  
predict specific mutations in the nucleotide-binding  
pocket or the predicted effector region which should  
5 lead to loss of function. Sequence analysis of unc-53  
alleles is instructive in determining which amino  
acids of UNC-53 are essential for function, e.g. as  
exemplified by the indication that an allele (n152)  
which has a differential effect on anterior versus  
10 posterior guidance has a deletion in a region of  
differential splicing. The differential splices of  
the C. elegans unc-53 gene encode different variants  
of the protein which independently affect posterior or  
anterior migration and/or cell specificity. One  
15 predicted exon in C. elegans unc-53 is indicated in  
Fig 1. It is conceivable that of two variants of the  
same protein one is inhibited or enhanced by a  
particular compound whereas the other is not (or to a  
lesser degree). Such a compound could then be used to  
20 control direction of migration or cell specificity by  
selective inhibition or enhancement.

(3) To develop pharmacological screens for inhibitors  
of a biochemical pathway a "gain of function"  
phenotype has been invented which can be expected to  
25 revert to wild type in the presence of specific  
inhibitors. Overexpression of UNC-53 in C. elegans  
myoblasts already leads to lethal subviable muscle  
phenotypes which can be easily scored with high  
throughput or a scorable heat shock inducible  
30 phenotype (Fig 21). They may form the basis for a  
pharmacological screen for inhibitors. A similar  
screen is obtained for over-expressing UNC-53 in  
mammalian cells. An alternative strategy is based on  
the homology to GTP binding proteins, RAS and dynamin  
35 and NTPases. We can introduce amino-acid changes in  
the nucleotide binding pocket which are

predicted/expected to lead to a constitutively activated or inactivated UNC-53. Similar changes are based on homologies with SOS, dynamin or ATP/GTP binding proteins from homology tables.

- 5 (4) Correct expression of UNC-53 in each cell line may be assessed by immunofluorescence and western blot analysis with the monoclonal antibody (mab) designated as 16-48-2.

10 The inventors have thus expressed and stably integrate the expression constructs in the neuronal, myoblast and 3T3 cell lines.

These cell lines are primarily used to:

- 15 - Assess the effect of UNC-53 expression on the morphology, motility, metastatic potential and growth cone extension of the cell lines.  
- Produce protein and mRNA  
- Screen for pharmacological compounds inhibiting observed UNC-53 mediated phenotypes  
20 - Analyse signal transduction pathways associated with UNC-53 activation (for example, phosphorylation,) - Immunofluorescence studies with mab 16-48-2 to assess changes in subcellular localisation following growth factor treatment.

25 Thus, the present invention provides for the identification of compounds which inhibit or enhance the UNC-53 signal transduction pathway. Such compounds can be used in the control of cell directional migration, motility and differentiation. These compounds are useful in the treatment of  
30 oncogenesis, psoriasis, neuronal degeneration and cell migration (metastasis).

The present invention also provides the ability to identify nucleic acid sequences and proteins which are involved in the UNC-53 pathway in C. elegans.

- 35 Such nucleic acid sequences and proteins may be UNC-53 equivalents, members of an UNC-53 pathway or may be

nucleic acid sequences or proteins which interact in the UNC-53 pathway, for example as demonstrated by the GRB-2/SEM-5 proteins. This knowledge of the UNC-53 pathway in C. elegans can be established as can factors which influence the functioning of the pathway, for example, factors/ proteins which feed into the pathway or are of a parallel pathway which at least, in vitro, compensates for steps in an UNC-53 pathway.

10       The identification of other components in the UNC-53 signal transduction pathway:

- (1) help to determine the interaction of UNC-53 with known signal transduction pathways (RAC-, RHO-, cdc42-RAS-pathway exchange factors, downstream or regulating kinases)
- 15       (2) identify the new interacting proteins which may constitute additional potential pharmacological targets.
- 20       (3) may assign functions to the more than 1000 amino acids of UNC-53 which have no homology to known proteins.

Accordingly, proteins which cross-react with anti-C. elegans UNC-53 protein antibodies can be isolated. The basic experiment protocol for purifying antigen-antibody complexes is described in Example 11. This system can also be used to identify factors which interact with proteins which bind to anti-UNC-53 C. elegans antibodies.

30       The following tissue sources may be used for immuno-precipitation:

- (1) Mammalian cells which exhibit a phenotype after transfection with unc-53 indicating that it interacts with vertebrate components of its signal transduction pathway.
- 35       (2) UNC-53 protein may be too low abundance to make affinity purification from wild type C. elegans

feasible. The inventors have affinity-purified UNC-53 from already constructed transgenic C. elegans lines which express UNC-53 under control of the hsp-16 promoter and/or the myosin promoter. These  
5 experiments in C. elegans are justified because with the vast amount of sequence information (genomic and cDNA) available, one has a good chance of identifying the corresponding genes in the databases with a minimum of peptide sequence.

10 Several types of proteins may be expected to co-purify with UNC-53, including GRB-2 and other proteins with SH3 domains of the Grb2 class or phosphorylation sites, RTK-receptors, subunits of an UNC-53 homo-heterodimer complex, downstream regulating kinases or  
15 proteins from the microfilament cytoskeleton.

This co-immuno-precipitation approach can also be used to dissect the order of events in this signal transduction pathway. For example: UNC-53 immuno-purified after stimulation of mammalian cell-lines  
20 with growth factors and pharmacological agents can also be assayed with respect to its state of phosphorylation, or complex formation with interacting proteins.

Proteins interacting with specific UNC-53 domains  
25 are identified using a yeast two-hybrid system, whereby two sets of hybrid proteins are used to assay for functional restoration of the GAL4 transcriptional activator: the first consisting of a GAL4 activation domain/UNC-53 structural domain of unknown function,  
30 the second derived from a cDNA library cloned into an expression vector to generate a library of hybrid proteins containing a GAL4 DNA binding domain. The yeast two-hybrid system is well known in the art.

A set of unc-53-fusion constructs can be  
35 constructed, including a fusion to  
(1) the full length protein,



- (2) the carboxyterminal domain (from second actin binding domain to the ATP/GTP binding domain),  
(3) The aminotermminus (predicted cortical localisation domain up to the SH3 binding sites),  
5 (4) a variety of overlapping constructs within the central domain of 1000 amino acids to which no function can as yet be assigned.

These are tested in yeast to exclude those which lead to activation of the reporter gene in the absence  
10 of the cDNA-activator fusion. cDNA libraries were transformed into these reporter strains and positive clones identified. (In this strategy, screening of multiple libraries requires very little effort (transformation followed by plating on selective and  
15 indicator medium)).

A preferred cDNA library is from cell lines in which a phenotypic change is observed following UNC-53 expression such as mouse N4 neuroblastoma cells or MCF-7 breast carcinoma cells. The yeast two hybrid  
20 system can identify interacting proteins or "sections" of nucleic acid which may not be translated in vivo but which may inhibit UNC-53.

Candidate positives are tested for the fusion-protein dependence of the reporter gene activation.  
25 The cDNA insert in remaining positive clones is sequenced. The obtained sequence is screened through the databases, which provides, especially in the case of C. elegans clones, significant extra sequence.

Another system also exists for the identification  
30 of proteins which bind or modify UNC-53. An UNC-53 protein is bound by conventional techniques to a column. A sample to be tested is then passed over the column. This sample may be fractions from cells from C.elegans, mammals or any other organism. These  
35 sample fractions may have been incubated with <sup>32</sup>ATP. In this course the "reaction" of the labelled fraction

with UNC-53 can be determined. If the UNC-53 on the column becomes  $^{32}\text{P}$  phosphorylated then this indicates that the sample fraction contains an UNC-53 modifying protein. Alternatively a constituent of the sample  
5 may bind to the UNC-53 and remain bound therewith on the column. The retention of any fraction of the sample on the column and the identification of the fraction can easily be determined by techniques known in the art.

10 Example 9 describes the identification of sensitive, dependant or resistant mutations as direct tools for the development of screens for compounds with similar or antagonistic activities. Both resistant and sensitising mutations may have a  
15 phenotype in the absence of the compound and no or a different phenotype in the presence of the compound. This permits the introduction of action-specificity in the screens.

High throughput screens are a basic feature of C.  
20 elegans genetic methodology. Non-complementation screens for new alleles in a locus require setting up of up to 8000 separate worm populations starting from one hand-picked individual each. This is done in 24 well plates or small Petri-plates. These are  
25 subsequently (after 1 or 2 generations) visually screened for a complex behavioural phenotype. For pharmacological screens where populations can be started from multiple individuals pipetted from a pool of synchronised eggs, high throughput screens can also  
30 be developed. If the endpoint of the assay can be scored in liquid, populations can be set up in microtitreplates. If the end-point is linked to a reporter gene (e.g.  $\beta$ -galactosidase activity) ELISA type colour-metric assays can be used to score the  
35 end-point. C. elegans can also be introduced into soils, exposed to compounds and subsequently recovered

and assayed. Such endpoints are used in the heat-shock assay developed by Stressgen (Stringham & Candido (1994), Environ. Toxicology and Chemistry, 13(8), 1211-1220).

5 Gain of function mutants of C. elegans or transgenic C. elegans in which a pathway of interest has been over- or constitutively activated, causing a dominant phenotype which can be used to develop specific screens for inhibitors.

10 Transgenic lines expressing UNC-53 ectopically under the C. elegans heat-shock (hsp-16) promoter, and body wall muscle (unc-54) promoter have been constructed. These lines lead to dominant phenotypes in development and are used directly to screen a  
15 spectrum of compounds. Where necessary or deemed useful endogenous C. elegans genes can be replaced by or complemented with human signal transduction pathways.

20 DEPOSITED CELL LINES AND PLASMIDS

	<u>STRAIN NAME</u>	<u>DATE OF DEPOSIT</u>	<u>LMBP ACCESSION NUMBER</u>
25	pTB54 Plasmid	22 MAY 1995	3296
	pTB112 Plasmid	22 MAY 1995	3295
30	pTB72	22 MAY 1996	3486
	TB4EX25 Cell Line	22 MAY 1995	1384 CB
35	TBAIn76 Cell Line	22 MAY 1995	1385 CB
40	HYBRIDOMA Cell Line	22 MAY 1995	1383 CB
	MCF-7 TRANSFECTED BREAST CARCINOMA		

	CELL LINE	24 MAY 1996	1550 CB
5	TRANSFECTED N4 NEUROBLASTOMA CELL LINE	24 MAY 1996	1549 CB
10	WILD TYPE MCF-7 BREAST CARCINOMA CELL LINE	24 MAY 1996	1551 CB

The above plasmids and cell-lines were deposited at the Belgian Coordinated Collections of Micro organisms (BCCM) at Laboratorium voor Moleculaire Biologie - Plasmidencollective (LMBP) B-9000, Ghent, Belgium, in accordance with the provisions of the Budapest Treaty of 28 April 1977.

The present invention will now be described with reference to the following Examples.

#### Examples

##### Example 1 - Molecular Characterisation of unc-53 gene in C. elegans

25

##### Screen for muscle pattern mutants :

C. elegans has two sets of muscles which are suitable to study this problem, the body wall muscles and the sex muscles. The sex muscles are a set of 16 muscle cells (4 muscle types) in the hermaphrodite and 41 cells in the male (10 muscle types) with distinct attachments points on the hypodermis and gonads. The sex muscles develop postembryonically and are not required for viability. The body wall muscles are arranged longitudinally (roughly 2 cells abreast) into four quadrants. At birth there are 81 cells. In postembryonic development, extra muscles interdigitate with these bringing the total number of body wall

muscles in the hermaphrodite to 95. Head, neck and body muscles can be distinguished within these rows on the basis of their innervation and patterning within the rows.

5           We have screened 4800 haploid genomes using Nomarski and polarized microscopy for mutants with specific attachment or pattern defects in a subset of the male sex muscles but with wild type body wall muscle pattern and myofilament organization, wild  
10 type movement and wild type male bursa anatomy (a sensitive indicator of wild type morphogenesis). Amongst the 21 identified mutants we selected for further study those with specific phenotypes in both the male and hermaphrodite sex muscles. As these  
15 muscles lie in different regions of the animals this was thought to reduce the chance that the male tail phenotype is a pleiotropic consequence of changes in regional identity of the tail or defects in male tail hypodermal lineage or morphogenesis.

20

Muscle phenotype of e2432.

Mutant e2432 was isolated on the basis of its phenotype in the male spicule retractor muscles, a pair of bilaterally symmetrical muscles which attach  
25 anteriorly to the body wall and posteriorly to the base of the spicules. The spicule retractors of mutant e2432 are shorter than wild type. Their attachment to the spicules is wild type, but their attachment point to the body wall is shifted posteriorly. The spicule  
30 protractors sometimes extend processes onto the attachment point of the spicule retractors on the hypodermis, suggesting the defect is not in these attachment points, but rather in the extension of the muscles towards that point. The diagonal muscles are  
35 in most specimens wild type but they are occasionally not parallel to one another or are have a dorsal

attachment point that is more ventrally positioned than in wild type. e2432 males have a nicely shaped fan with the normal pattern of rays, suggesting that the sex muscle defect is not pleiotropic due to defects in the hypodermis.

e2432 hermaphrodites have a reduced ability to lay eggs which is variable from animal to animal. This is due to a muscle pattern defect in the vulval sex muscles. The uterine muscles, 8 muscle cells which circle the hermaphrodite uterus, are wild type in e2432. The vulval muscles are a set of 4 pairs of cells arranged symmetrically in a cross-pattern around the vulval slit. Each pair consists of one vm1 and one vm2 muscle cell. The vm2 muscles attach to the junction between uterus and vulva and extend anteriorly to attach to the hypodermis in between two muscle cells of the ventral body wall muscle quadrant. In e2432 these muscles are shorter than in wild type small. In e2432 they can only be visualized by laser confocal microscopy (after FITC-phalloidin staining of the myofilaments). This showed that they attached to the uterus as in wild type, but that their attachment to the body wall is ectopic (in a random position lateral of the vulva, usually on the ventral edge of the muscle row). In e2432 vm2 myofilaments are oriented more dorsoventrally than in wild type (where their orientation is essentially in the longitudinal axis of the animal). This phenotype is not due to a defect in the attachment point on the epidermis to which these cells should attach in wild type, since we frequently observe that the vm1 sex muscles make an apparently wild type attachment to this unoccupied attachment point.

In wild type hermaphrodites, the vm1 muscle cells attach close to the junction between epidermis and vulva and in the adult extend dorsally and anteriorly

(under an angle of 45-50 degrees with respect of the vulval slit) to attach to the hypodermis at the dorsal edge of the ventral body wall muscle quadrants. In e2432 the attachment of the vm1 muscles to the vulva is wild type. With their other end they attach, like wild type vm1 cells, along the dorsal of the edge of the ventral body wall muscles. However the angle between the vulval slit and the myofilaments of the vm1 sex muscles is reduced (less than 45 degrees) so that their dorsal attachment point is closer to the vulva than in wild type. The forces acting on the vulva can be separated in an antero-posterior and a dorsal vector. In e2432, the antero-posterior vector of both the vm1 and vm2 muscle is significantly reduced, leading to a reduced ability to open the vulva upon contraction. Studies in which vulval muscles were ablated individually or in groups suggested that 2 vulval muscle cells of wild type orientation are sufficient for wild type function.

Adult C. elegans hermaphrodites have 95 body wall muscle cells arranged longitudinally (roughly 2 cells abreast) into four quadrants. In wild type cells these cells are spindle shaped.

e2432 adults have body wall muscles with a wild type muscle cell and myofilament pattern, except that cells with interdigitating tips occur more frequently than in wild type. Like the unc-53 phenotype in the male and hermaphrodite sex muscles, this body wall muscle defect, which can also be observed in other guidance and attachment mutants like unc-6 and mups, can also be attributed to a reduced ability to extend "growth cones" otherwise referred to as cell processes in the anterior-posterior axis of the animal.

Position on the genetic map :

e2432 was mapped to the left arm of chromosome II

and was found not to complement unc-53(e404). The unc-53 locus was originally identified by Brenner (1974), Genetics, 77, 71-94 as one of the uncoordinated mutants but has received only sporadic attention in general phenotypic surveys of the UNC-collection (Hedgecock et al (1987), Development, 100, 365-382 and Siddiqui (1990), Neurosci. Res. (Suppl) 13, 171-190, in a genome wide screen for egg laying defective mutants (Trent and Horvitz (1983), Genetics, 104, 619-647) and using e2432 as a tool to study the effect of body shape on the pattern of neuronal processes (Hekimi and Kershaw (1993), J. Neuroscience, 13(10) 4254-4271). We initiated a detailed genetic and phenotypic analysis of this locus using the existing available alleles which various colleagues isolated in different screens : The canonical unc-53 allele e404, a strong UNC was isolated by Sydney Brenner. Alleles n152, n166 and n1199 have been obtained in screens for egg laying defective mutants. Alleles NJ234 and NJ222 were isolated by Ed Hedgecock in a screen defective in excretory canal outgrowth. As these screens were aimed at isolating viable fertile alleles, we isolated additional alleles by pre-complementation screens designed to yield loss of function alleles irrespective of their phenotype. e2432/mnDf90 hermaphrodites are egl, weak unc's with a slightly stronger phenotype than e2432. Matings were set up on 3 cm petri dishes between 2 to 3 unc-53(e2432) sqt-1(sc13) /+ males and 2 e2431ts or dpy-6(e14) hermaphrodites mutagenized with EMS in the L4 stage (Brenner, 1974) , Genetics, 77 71-94. The F1 egl, unc-53 like hermaphrodites, which may be unc-53(e2432) sqt-1(sc13)/unc-53(new) were cloned on petri dishes and their offspring examined for the segregation of new unc-53 alleles. In two screens, two unc-53 alleles, 5 and 8 were isolated in an estimated 13000



F1 offspring, giving an approx. mutation rate 1/3250 mutagenized chromosomes. Sqt-1(sc13), an allele of sqt-1 that confers a roller phenotype was included because it is closely linked to unc-53 (0.2 m.u.) and marks the original allele e2432. e2431ts, an X-linked ts larval lethal with a mup phenotype was included to eliminate F1 hermaphrodites arising from selfing and F1 males which can mate. In the second screen dpy-6(e14) was included to prevent F1 males from mating with F1 hermaphrodites.

All unc-53 alleles used in this study fail to complement to e2432. Complementation was tested by mating unc-53(e2432) sqt-1(sc13)/+ males to hermaphrodites of the respective alleles. The male sex muscle phenotype described above for e2432 was found to be the only 100% penetrant phenotype in the unc-53 locus (see below) and was the primary phenotype used in complementation tests. Each of these alleles was also complemented to mnDf90 by mating unc-4 mnDf90/mnC1 males to unc-53 homozygotes and temporary unc-53/unc-4 mnDf90 lines were established to evaluate the phenotype. The male and hermaphrodite phenotypes of all alleles over deficiency is identical or slightly, but not substantially stronger than that of the homozygous lines (which is not unusual for a large deficiency).

S. Brenner mapped unc-53 to 2.9 +/- 0.7 map units from dpy-10 (chromosome II). We refined this map position by mapping unc-53 with respect to different deficiencies in the region and doing three factor crosses between unc-4 and sqt-1, a 1.5 map unit interval. Unc-53(e2432)/+ males were mated in unc-4 sqt-1 hermaphrodites. Non-rolling F1 offspring were cloned on petriplates and their broods screened for the segregation of unc-53(e2432). Unc-4 non sqt-1 and sqt-1 non unc-4 hermaphrodites were picked from those

plates and cloned on petriplates. 6 out of 42 *sqt-1* non *unc-4* recombinants segregated *unc-53* and 3 out of 18 *unc-4* non *sqt-1* recombinants did not segregate *unc-53*. This yields a relative position of *unc-4* / 51 / *unc-53* / 9 / *sqt-1*. Or a calculated map position for *unc-53* on chromosome II, 0.23 map units left of *sqt-1*.

*Unc-53* (e2432) was mapped relative to three deficiencies in the region *mnDf90 mnDf87* and *mnDf77* by mating e2432/+ males to *unc-4 Dfx/mnC1* hermaphrodites and scoring for males and hermaphrodites with the *unc-53* phenotype in the F1. The experiment was also performed by mating *unc-4 mnDfx/mnC1* males to homozygous *unc-53. mnDf87* and *mnDf90* do not complement *unc-53* while *mnDf77* complements *unc-53*. *Ooc-3*, the only other gene on the genetic map in the region, was found to complement *unc-53* in identical crosses between e2432 and *unc-4 ooc-3/mnC1*. Further mapping of *unc-53* relative to RFLPs between wt strains in the region and the molecular cloning confirmed the map position of *unc-53* (see below).

#### Molecular characterization :

We started cloning the *unc-53* locus because the study and interpretation of the *unc-53* phenotype and the different mutants in the locus would be greatly facilitated by having information on and probes for the *unc-53* mRNA and gene product.

At the time we initiated cloning of *unc-53*, a contig extending between *unc-4* and *sqt-1* (approx. 1500 kb) had been identified by A. Coulson and J. Sulston (*C. elegans* genome project LMB Cambridge), with no clone markers in between. To correlate the genetic map with the physical map in this region we positioned cosmids of this contig relative to the deficiencies *mnDf77*, *mnD87* and *mnDf90* by comparing band intensities of Southern blots of *mnDfx/mnC1* strains probed with

- 53 -

cosmids throughout the region. Cosmid KO2F7 is deleted in mnDf90 but not deleted in mnDf87 and mnDf77 thus identifying a leftmost location for unc-53. Cosmids W10G4, T08D11 and F33G3 are in the unc-53 region (not deleted in mnDf77 but deleted in mnDf87 and mnDf90). Cosmid KO4H9 is deleted in mnDf77 and identifies a rightmost location for the gene. The distance between KO2F7 and KO4H9 is approx. 10 cosmids.

10 To narrow down the position of unc-53 further we looked for restriction fragment length polymorphisms between wild type strains in this interval and identified N2/RC301 RFLPs in cosmids W10G4, F40F8 and F22G3. We mapped these using three factor crosses with  
15 the strains unc-53 sqt-1/RC301 and unc-4 unc-53/RC301. We mapped F22G3 and F40F8 between unc-53 and sqt-1 at the following relative distances :  
unc-4 / 9 / W10G4 / 2 / unc-53 / 1 / F40F8 / 1 / F22G3 / sqt-1.

20 These data localize unc-53 in an interval of approx. 80kb in which more than 15 differently overlapping cosmids are available. Pools of cosmids were injected in unc-53(n152) gonads together with the rol-  
25 6 selectable marker. Transient roller lines were established and scored for rescue of the unc-53 phenotype. Cosmid T28D2 was found to rescue the backward movement egg laying phenotypes of allele n152 .

30 A genomic library of N2 in lambda 2001 was screened with T28D2 and flanking overlapping cosmids. These were assayed in pools and individually for transformation rescue. Lambda clone, S4 carrying a sixteen kb insert was shown to give some rescue  
35 activity. Using restriction fragments of S4 as a probe, cDNA clones M5 (3.8 kb) and M18 (1-2 kb) were

isolated from a Lamda MGU1 cDNA library. Both M18 and M5 contain an identical 3'-end as judged by restriction fragment analysis. Partial sequence analysis showed that M18 is shorter version of M5.

5 Insert M5 was sequenced on both strands and was found not to be a poly-A tail at its 3'-end but appears not to full length at its 5'-end.

To find the 5' end of the unc-53 transcript we did nested PCR on L2 stage random primed cDNA, between  
10 antisense oligos tab2 and tab (43 bp away from the 5' end of cDNA M5) and an oligo to the SL1 trans-spliced leader sequence. This sequence is transspliced to the 5'-end of most C. elegans mRNAs. This yielded at least 6 classes of PCR-fragments which have been subcloned  
15 and sequenced. All contain the 43 bp between oligo tab2 and the 5' end of cDNA M5 (bp1281 to 1338).

The longest PCR fragment (TB3) extends the sequence of cDNA M5 with 1280 bp. When added to the length of the cDNA M5, this unc-53 transcript which we constructed  
20 in vitro and named tb3-M5 would then be 5073 bp long (including some poly-A tail) and have a 1528 AA open reading frame. Recently a 5 kb cDNA, was identified in an embryonic cDNA library which has the TB3-5'-end (including part of the SL1), and the same 3'-end as  
25 M5, suggesting that TB3-M5 occurs in vivo. Similar PCR reactions in which the SL1 oligo was replaced by an SL2 transplice oligo gave no reaction products. Preliminary Northern blot analysis identifies a major 5.0 kb transcript and at least 2 smaller transcripts  
30 that are expressed in L2, L4 and adult worms.

It needs to be examined whether the unc-53 5' ends reported here are made in vivo and encode different proteins or whether they represent PCR noise. The smaller PCR-fragments TB1b, TB16, TB1, TB6b and TB22  
35 are "nested deletions" of clone TB3 with SL1's at their 5' end. The sequence of each is identical in the

regions of overlap. The shorter SL1 transspliced transcripts contain ATGs downstream of the SL1 addition sites at positions 466, 988 and 1324. Comparison to the sequence of genomic clones confirmed  
5 that the SL1s are spliced onto intron exon boundaries. However not all intron-exon boundaries receive SL1, suggesting that there is some specificity to this differential trans-splicing.

Recently the C. elegans sequencing consortium has  
10 sequenced cosmids F45E10. We mapped cDNA tb3-M5 onto these cosmids and found that unc-53 is an unusually large locus. It has 23 exons spread over more than 31 kb of genomic DNA.

The lambda clone S4 that rescues does not contain  
15 the first 430 bp of the unc-53 transcript. This suggests that the ORF between positions 63 and 430 is not essential for transformation rescue. This rescue may derive from expression of transcripts TB6b or TB22 or from "non-specific" initiation of transcription on  
20 the extrachromosomal arrays.

Additional confirmation that M5 was derived from the unc-53 transcription unit is provided by the observation that allele n152 has a 300 bp deletion, disrupting the sequence of cDNA M5 and leading to a  
25 large (possibly complete) reduction of UNC-53 protein in n152 embryos stained in immunofluorescence with an anti-unc-53 antibody (16-48-2). In addition, allele e2432 was found to carry a 3-4 kb insertion in this transcription unit.

30

Sequence homology :

Antibody staining :

The NdeI-EcoRI fragment of cDNA M5, the 47 kd  
35 fragment of UNC-53 encoded by the NdeI-EcoRI (position 3187 to 4458 (tb-M5 fig 3) protein sequence

fig 2) was subcloned in the T7 expression vector  
prk172 (yielding vector TB66 and expressed in E. coli.  
Inclusion bodies containing recombinant protein were  
purified, by processes known in the art solubilized in  
5 8 M Urea and the recombinant protein purified over a  
DEAE column equilibrated in 8M urea. Purified protein  
was mixed with complete Freund's adjuvant and injected  
in a rabbit and 4 Lou rats. This was followed six  
weeks later by bi-weekly boosts with antigen mixed  
10 with incomplete adjuvant. All sera are active in  
western blotting at titers of 1:30,000 on Western  
blots of the 47 kd unc-53 fragment expressed in  
E.coli. With this western blotting assay, a rat-  
mouse hybridoma cell line was prepared producing a  
15 monoclonal antibody to UNC-53. Mab 16-48-2 has the  
following properties :  
- protein G-binding  
- binding activity on western blots of  
(1) the 47 kd UNC-53 fragment expressed in E. coli,  
20 (pTB66)  
(2) the 57 kd carboxyterminal fragment of UNC-53  
expressed in E. coli (construct pTB65.)  
(3) the full length TB3-M5 UNC-53 expressed in E.  
coli (construct pTB61) and mammalian cells (COS-cells;  
25 constructs pTB54 and 56).  
- immunoprecipitation of native and SDS denatured full  
length TB3-M5 UNC-53 construct pTB50 expressed in  
vitro-transcription translation reactions in  
reticulocyte lysates.  
30 - immuno-histochemistry in wild-type C. elegans fixed  
with methanol, acetone or paraformaldehyde and  
transgenic C. elegans expressing UNC-53 tb3-m5 pTB110,  
111 or 112 in epidermis, neurones, gut and muscle.  
Mab 16-48-2 fail to detect antigen of the correct  
35 size on Western blots of total worm proteins or worm  
proteins fractioned by progressive extraction with

detergents, urea and SDS.

Excretory canal phenotype :

5 The excretory canal of C. elegans is a large H-shaped cell. Its cell body is positioned ventrally at the level of the pharyngeal bulb and send out two processes dorsally. At the level of the lateral epidermis (seam) each of these bifurcates and extends anteriorly and posteriorly over the seam cells, until  
10 they extend over most of the whole body length. It has been reported that in unc-53 the posterior process of the excretory cell does not extend up to the V6/T seam-cell boundary (E. Hedgecock et al., (1987), Development, 100 365-382).

15 We have done an extensive characterization of this phenotype in all alleles listed, either by direct in vivo Nomarski microscopy or UL6 rol6d marked unc-53 strains which express LacZ in the epidermis and excretory cell (Hope(1991) Development 113(2) 399-  
20 408). In wild type the excretory cell processes are straight. In unc-53 the canal is often meandering from left to right over the seam before it arrests prematurely, as if it has lost directional cues in its migration. It never leaves the lateral epidermis  
25 seam. Both the anterior and posteriorward processes are affected.

In weak unc-53 alleles the posterior excretory canal processes arrest anywhere between the vulval region and the V6/T boundary. We noticed that in even  
30 the strongest alleles or in unc-53/Df heterozygotes the canal arrests unusually frequently at or close to the vulva and never substantially before the vulva . We therefore set out to test whether the gonad dependent attractive signal which attracts the sex  
35 myoblasts to the gonad also might attract the excretory canal in an unc-53 independent manner to the

vulval region. If this is the case we would expect that in a strong *unc-53* mutant *n152* in which the 2 somatic gonad cells (the source of the signal) have been ablated, the excretory canal migration would be fully arrested. As a control we ablated one germ cell and one somatic gonad cell (Z1 and Z2 or Z2 and Z4). Embryos were ablated in the comma to 2 fold stage and the position of the excretory canal scored double blind in hatched embryos. At the time of ablation, the canal may already have started growing out. At hatching, the endpoint of our experiment, the growth cone of the posterior canal process has reached just beyond the gonad. Although these are technically difficult laser ablations, the results show a substantial difference in excretory canal outgrowth between embryo with an ablated somatic gonad and control ablated embryos. In the experimental series the canal usually arrested a significant distance from the gonad or any other potentially damaged cells, suggesting the loss of a long range signal as described for the SM myoblast migration (Thomas *et al* (1990) and Stern (1991)). In the control series the excretory canal usually extended as far as unablated *n152* and into region of the partially ablated gonad. This indicates that the premature arrest observed in the experimental series was not due to encountering a damaged region.

A gonad dependent and independent pathway were found to act redundantly in the posteriorward migration of the sex myoblasts. The data suggest that in wild type the migration of excretory cell growth cones is also guided by a gonad dependent and a gonad independent cue. In both cases the gonad dependent cue acts towards the gonad, but from opposite directions. However the gonad independent signal act anteriorward on the SM myoblasts and posteriorward on



the posterior excretory cell growth cones. Since single mutants in both the gonad dependent pathway (sem-5) and independent pathway (unc-53) have no excretory cell phenotype these pathways may be  
5 redundant in the trajectory up to the gonad. An analogous redundancy has been observed for the sex myoblast migration. In the trajectory between gonad and tail the gonad independent pathway acts in different directions on the SM cells versus the  
10 excretory cell. In the excretory cell it acts in both anteriorward and posteriorward migration. A simple explanation which is elaborated in detail below is that unc-53 (like sem-5) may act downstream of a variety of receptors interpreting different cues.

15 The previously described interaction between the gonad and the sex myoblasts was rationalizable as an interaction between cells due to become part of the same organ. The interaction between the excretory cell and the gonad we report here suggests that the gonad  
20 may have a more general role as organizer cell : migrations in the embryo. We wish to point out that the described dependent and independent pathways are formal genetic concepts. It is for example possible that in unc-53 embryos or unc-53 embryos in which the  
25 gonad dependent pathway has been genetically or laser ablated, as yet to be identified, pathway defining growth cones are misplaced leading indirectly to defective sex myoblast, neuronal (PLM, see below) or  
30 excretory canal migration. The observed highly restricted expression of unc-53 is an additional indication of this possibility.

#### Sex muscle phenotype :

35 All unc-53 alleles exhibit the sex muscle phenotype described for e2432. We quantified phenotype

in eight alleles :

Young adults grown at 20°C were mounted for polarized light or Nomarski microscopy on 2% agarose pads containing 0.2% phenoxypropanol as described in Sulston and Horvitz (1977) Dev. Biol. 56, 110-156 . The vm1 sex muscles were examined under polarized light with a 40x objective and a Brace Kohler compensator and photographed. In addition, adults were fixed, incubated with fitc-coupled phalloidin and mounted for fluorescence microscopy as described in Goh and Bogaert (1991) Dev. Biol. 56, 110-156. The angle between the longitudinal axis of the animal and the central bundle of myofilaments of the anterior and posterior vm1 was measured from the negatives with a protractor. As the vulva is a transverse slit at a right angle to the cylindrical body axis, the angle between the vm1 and the vulval slit can be measured independently of which side of the animal faces the observer.

20

Neuronal phenotype :

Unc-53 animals move poorly backwards when prodded but has good forward movement (Brenner (1974) Genetics 77 71-94). Various aspects of the neuronal phenotype of unc-53 has been reported in general phenotypic surveys of the UNC-collection (Brenner (1974) Genetics 77 71-94). : The posterior branch of the PDE neuron can be abnormal ( Hedgecock et al. (1987) Development 100 365-382) and the mechanosensory PLMR & PLML neurons can have commissures into the ventral cord at a position much posterior than in the wild-type. There are also frequently multiple ventralward PLM commissures evenly spaced along the posterior half of the body (Siddiqui (1990) Neurosci. Res. (Suppl) 13 171-190), Hedgecock et al., (1987) Development 100 365-382).

35

Examples 2 to 5 - Biochemical Analysis of UNC-53Example 2 - Immunoprecipitations of <sup>35</sup>S labelled unc-53 gene products.

5

The rat anti-UNC-53 monoclonal antibody, 16-48-2 (obtained from the hybridoma LMBP Accession no. 1383CB) elicited against a 47 kD fragment of the 3' end of UNC-53 from C. elegans was used to immunoprecipitate UNC-53 proteins. In this experiment, the full length unc-53 construct pTB50 (Fig. 11) was transcribed and translated in vitro in rabbit reticulocyte lysates. The resulting radioactively labelled <sup>35</sup>S unc-53 gene products were incubated with the monoclonal antibody under both denaturing (using SDS) and non-denaturing conditions, then incubated with protein G sepharose. The bound products were analysed by SDS-PAGE and fluorography. Monoclonal antibody 16-48-2 recognised both native and SDS denatured radioactive UNC-53 products verifying that the protein translated in vitro was bona fide UNC-53. This result shows that immuno-precipitation is a useful tool in schemes to purify native protein and to identify UNC-53 protein complexes in biochemical experiments.

25

Example 3 - Actin sedimentation assays (8A variant).

30

Besides the N-terminal region of the protein which is similar to actin binding proteins, the predicted protein sequence of UNC-53 identified two putative actin binding sites. The first borders on the 3' end of the region of  $\alpha$ -actinin/ $\beta$ -spectrin homology and the second lies in the 3' end of the cDNA sequence. This suggests that UNC-53 could potentially

35

bind two actin molecules and via actin cross-linking, stabilise a particular growth cone spike to promote directional extension. Alternatively, the two actin binding sites may serve to anchor UNC-53 (and its shorter gene products) to the microfilament cytoskeleton to then transduce a signal via the NTPase domain to the downstream pathway.

To test the two site model, full length and truncated versions of UNC-53 (pTB50 and pTB52) were transcribed and translated in rabbit reticulocyte lysates for 90 minutes following standard protocols (Promega). To remove insoluble components, the reactions were airfuged for 1 hour at 100,000 x g and the supernatant containing <sup>35</sup>S labelled UNC-53 products introduced in actin co-sedimentation assays according to the method of Vancompernelle et al. (1992), EMBO J. 11, 4739-4746. In this procedure, radioactively labelled UNC-53 was incubated with monomeric G-actin in G buffer (2 mM Tris pH 7.5, 0.2 mM CaCl<sub>2</sub>, 0.5 mM  $\beta$ -mercaptoethanol, 0.2 mM ATP) for one hour at room temperature. The salt concentration was then increased with F buffer (1 M KCl, 10 mM MgCl<sub>2</sub>) to a final concentration of 100 mM to promote polymerisation of G-actin to F-actin. After an additional one hour incubation, polymerised F-actin/protein complexes were pelleted at 100,000 x g in an airfuge, washed with G buffer, resuspended in Laemmli buffer and separated by denaturing SDS-PAGE. The presence of actin in the pellets was confirmed by Coomassie staining while radioactively labelled UNC-53 products were detected by fluorography. Both the full length UNC-53 protein, pTB50, and the truncated construct, pTB52 translated in vitro in rabbit reticulocyte lysates cosedimented with F-actin at starting G-actin concentrations of 50-100  $\mu$ g/ml. This suggests that UNC-53 binds to microfilament

cytoskeleton. Moreover, deletion of the first putative actin binding site (pTB52) did not eliminate actin binding.

5     Example 4 - UNC53 interacts with F-actin cytoskeleton  
          (7A and 8A variant)

          Analysis of the predicted protein sequence of  
UNC-53 identified two putative actin binding sites of  
10    the LKK class. The first borders the 3' end of the  
      region of  $\alpha$ -actinin/ $\beta$ -spectrin homology in the amino  
      terminus of the protein while the second lies in the  
      3' end of the protein sequence upstream of the  
      putative nucleotide binding domain. A single UNC-53  
15    monomer could thus potentially bind and crosslink two  
      actin molecules.

          To test whether UNC-53 associates with the actin  
cytoskeleton, a 7A (pTB72) and 8A version (pTB73) of  
unc-53 (Figures 25 and 27 respectively) were  
20    transcribed and translated in rabbit reticulocyte  
      lysates and the  $^{35}$ S labelled products introduced into  
      F-actin co-sedimentation assays (Figure 35a). The  
      full length UNC-53 protein (pTB72) translated *in vitro*  
      cosedimented with F-actin at starting G-actin  
25    concentrations of 100 mg/ml (Figure 35b) suggesting  
      that UNC-53 interacts with F-actin. By 250 mg/ml, all  
      of the UNC53 protein co-sedimented with the F-actin  
      pellet. In contrast, no UNC53 was present in the  
      pellet of the control reaction without actin. Thus,  
30    sedimentation was purely actin dependent. This result  
      also indicated that the *in vitro* UNC-53 protein  
      remained soluble even after the salt concentration was  
      raised.

          Deletion of the first putative actin binding site

in pTB73 did not eliminate actin binding since the larger pTB73 products, including the largest fragment co-sedimented with F-actin under the identical set of conditions (Figure 35b). However, since the rabbit  
5 reticulocyte lysates contain numerous proteins, it is possible that the interaction of UNC-53 with actin may not be direct but rather mediated through another associated protein.

Several smaller radiolabelled protein fragments  
10 in the TnT reactions were observed in addition to the predicted protein products. Immunoprecipitation experiments confirmed that these products were UNC53 derived. Most likely they result from additional translational starts at internal methionines, since  
15 the identical set of smaller products was observed from reaction to reaction; or from premature termination and proteolytic degradation. Many of these smaller fragments also co-sedimented with F-  
actin. Since the second predicted actin binding site  
20 is within the 3' end of the molecule, truncated proteins that are the result of internal starts would be expected to have this site and to bind actin.

#### EXPERIMENTAL PROCEDURES:

25 Construction of UNC53 plasmids.

The complete unc53 cDNA was cloned as a 5.1 kb NotI-ApaI cassette in the mammalian expression vector pCDNA3 (Invitrogen) to generate plasmid pTB72, the 7A clone variant. To optimize translational initiation  
30 at the first methionine, a mammalian KOZAK consensus sequence was engineered upstream of the start methionine by PCR amplification of DNA coding for the first 139 amino acids of the amino terminus with the

oligonucleotides BG03 (5'-  
ataagaatgcggccgcccgcacgtcaaattgtagaattgata-3')  
and BG02 (5'-cgcggtatcctcaaaccgcgggtggcataatggatg-3').  
BG03 contains the mammalian KOZAK consensus sequence  
5 in addition to a NotI restriction site. pTB73 is a  
deletion of the first 408 base pairs of the unc53  
cDNA contained in the vector Bluescript II-KS. This  
construction removes the first two methionines of the  
unc53 cDNA sequence such that the first possible start  
10 methionine in pTB73 is at amino acid position 165 in  
the cDNA sequence. In all these constructs, (pTB72,  
pTB73 and pTB50) the unc53 cDNA is inserted into the  
multiple cloning site such that the T7 promoter is  
immediately upstream of the 5' end of the cDNA  
15 sequence.

The first 139 amino acids of the UNC53 cDNA were  
amplified by PCR with oligonucleotides BG01  
(5'ggaattccaaccatatgacgacgtcaaattgtagaattgaata-3') and  
BG02 (5'-cgcggtatcctcaaaccgcgggtggcataatggatg-3') to  
20 generate a convenient NdeI cloning site immediately  
upstream of the start methionine. This amplification  
was cloned as an NdeI-BamHI fragment into the  
prokaryotic expression vector pRK172 (Godedert M. and  
Jakes R. (1990), EMBO J. Vol. 9, pp 4225-4230 and  
25 McLeod M et al, 1987 EMBO. J. Vol 6, pp 729-736) to  
generate construct pTB57. pTB61 contains the PCR  
derived amino terminus of pTB57 in addition to the 3'  
end of pTB50. Thus pTB61 contains the identical unc53  
8A variant cDNA as in pTB50, but as an NdeI-NcoI  
30 fragment in the vector pRK172 for prokaryotic  
expression.

In vitro transcription/ translation reactions

The UNC53 cDNA constructs pTB72, pTB73 or pTB50 were transcribed and translated for 90' at 30°C in a cell free T7 polymerase expression system in rabbit reticulocyte lysates following the company's protocols (ProMega). Prior to further manipulations, the reactions were centrifuged for 1 hour at 100,000 x g to remove insoluble components. In all subsequent experiments, the supernatant containing the soluble fraction of <sup>35</sup>S labelled UNC-53 products was utilized.

#### 10 Actin co-sedimentation assays

Soluble radioactively labelled <sup>35</sup>S-Met-UNC53 products were introduced in actin co-sedimentation assays according to the method of Vancompernelle et al. (1992). In this procedure, radioactively labelled UNC-53 was incubated with monomeric G-actin in G buffer (2 mM Tris-pH 7.5, 0.2 mM CaCl<sub>2</sub>, 0.5 mM β-mercaptoethanol, 0.2 mM ATP ) for one hour at room temperature and then the salt concentration increased with F buffer (1 M KCl, 10 mM MgCl<sub>2</sub>) to a final concentration of 100 mM to promote polymerization of G-actin to F-actin. After an additional one hour incubation, polymerized F-actin/protein complexes were pelleted at 100,000 x g in an airfuge (Beckman), washed with G buffer, resuspended in Laemmli buffer and separated by denaturing SDS-PAGE. The presence of actin in the pellets was confirmed by Coomassie staining while radioactively labelled UNC-53 products were detected by fluorography. Briefly, after destaining, gels were soaked in 45 % methanol, 7.5 % acetic acid (vol/vol) for 30 minutes, followed by 30 min. in dimethyl sulfoxide (DMSO), and 1 hour in 10 % PPO dissolved in DMSO (wt/vol). The scintillant was precipitated by rehydrating the gels with four five



minute water washes. After drying, gels were exposed to Xray film (Hyperfilm-Amersham).

#### Immunoprecipitations

5           To confirm that the radioactively labelled proteins translated *in vitro* were of UNC53 origin, an anti-rat monoclonal antibody, 16-48-2, elicited against a 47 kD fragment of the 3' end of UNC-53 was used to immunoprecipitate UNC-53 proteins. In this  
10 experiment, the unc-53 construct pTB50 was transcribed and translated *in vitro* in rabbit reticulocyte lysates. The resulting radioactively labelled <sup>35</sup>S UNC-53 gene products were incubated with the monoclonal antibody under both denaturing (0.4% SDS, 2.0% Triton  
15 X-100) and non-denaturing conditions for 1 hour at room temperature, then incubated with protein G sepharose for 2 hours at room temperature, the beads washed 3 times with PBS and the bound products analyzed by SDS-PAGE and fluorography. Monoclonal  
20 antibody 16-48-2 recognized both native and denatured radioactive UNC-53 products. As a control, a reaction without monoclonal antibody 16-48-2 was treated identically.

#### 25   Example 5 - Interaction of UNC-53 with SEM-5/GRB-2

          The observation that certain alleles of UNC-53 enhance the sex myoblast migration defect of sem-5 mutants is difficult to interpret. While the genetics  
30 suggests that UNC-53 and SEM-5 cooperate to regulate sex myoblast migration, it is unclear whether this is the result of a direct molecular interaction. To answer this question, two types of biochemical experiments were used to determine if UNC-53

physically interacts with SEM-5. In the first experiment, radioactively labelled  $^{35}\text{S}$  UNC-53, synthesised in reticulocyte lysates, was incubated with SEM-5/GST (glutathione-S-transferase) fusion protein bound to glutathione resin or with GST protein bound to glutathione resin. After incubation, the beads were washed and the bound proteins analysed by SDS-PAGE and fluorography. This demonstrated that UNC-53 made in vitro specifically bound to the SEM-5/GST fusion protein resin. The GST fusion proteins have been previously described. Purification of GST-fusion proteins was facilitated by using a commercially available kit (Pharmacia). All purification methods followed the manufacturer's protocols.

To further characterise the nature of the interaction with SEM-5, a second experiment utilised Western blot overlays. UNC-53 fusion proteins were expressed in E.coli and the denatured protein lysates separated by SDS-PAGE and blotted to Immobilon-P nylon membrane (Milipore). Blots were incubated with biotin labelled SEM-5/GST, GRB-2/GST or GST protein, washed and bound multi-protein biotinylated complexes detected by probing with an avidin-alkaline phosphatase conjugate. The results from this experiment demonstrated that SEM-5 and its mammalian homologue GRB2 can interact with UNC-53 in vitro. Binding was observed in induced cell lysates only and probing with the UNC-53 monoclonal antibody 16-48-2 detected the identical sets of products. In addition, only the full length UNC-53 fusion, pTB61 (Fig. 7), which contained the SH3 binding sites gave a positive result (pTB52 was not tested) No signal was detectable for either of the SH3 binding site minus fusion proteins, pTB57 (Fig. 11) or pTB65 (Fig. 11). This provides supportive evidence that the polyproline

repeats of the UNC-53 directly bind to the SH3 domains of SEM-5. Moreover, these results show that a SEM-5 or GRB-2/GST glutathione resin may be used in schemes to affinity purify native UNC-53 from tissue culture  
5 cells or nematodes or other organism extracts.

#### Detailed Methodology

Radioactively labelled  $^{35}\text{S}$  UNC-53 synthesized in reticulocyte lysates was incubated with SEM-5/GST  
10 (glutathione-S-transferase) fusion protein bound to glutathione resin or with GST protein alone bound to glutathione resin for one hour at 20°C. After incubation, the beads were washed four times with Phosphate Buffered Saline (PBS)/Triton X-100 (0.2%)  
15 and the bound proteins analyzed by SDS-PAGE and fluorography. The SEM5 and GRB2 GST fusions have been previously described (Lowenstein et al., 1992; Stern et al., 1993). Purification of GST-fusion proteins was facilitated using a commercially available kit  
20 (Pharmacia). All purification methods followed the company protocols.

#### Western blot overlays

Approximately 500-1000 mg each of purified GRB2-GST protein or GST protein were biotin labelled by the  
25 following procedure. After overnight dialysis in PBS at 4°C, 1 M Hepes, pH7.4, was added to a final concentration of 100 mM and 50-100 mg of biotinylation reagent, dissolved in dimethyl sulfoxide, and the mixture incubated at 20°C for 90 minutes. The  
30 biotinylation reaction was stopped by the addition of 1 M Tris, pH7.4 to a final concentration of 100 mM and the labelled proteins stored on ice.

The UNC-53 construct pTB61 was expressed in *E. coli* strain BL21 (DE3), and the denatured protein

lysate separated by SDS-PAGE and electroblotted to Immobilon-P nylon membrane (Millipore). Membranes were blocked with 1 % skim milk powder in TBS-T (20 mM Tris, pH7.6; 0.14 M NaCl; 0.1% Tween-20) for 1 hour at 37°C. Subsequently, membranes were incubated in equimolar amounts of either biotin labelled GRB-2/GST or biotin labelled GST protein for 1 hour at 20°C, washed 4 x with TBS-T and bound multi-protein biotinylated complexes detected by probing for 1 hour at 20°C with an avidin-alkaline phosphatase conjugate (dilution 1:5000). Biotinylated protein conjugate complexes were visualized with a chromogenic solution containing bromochloroindolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) in 100 mM Tris(pH 9.5), 100 mM NaCl, 5 mM MgCl<sub>2</sub>. Development was terminated with 10 mM Tris (pH8.0), 1 mM EDTA.

#### Example 6 - Transgenic Analysis

To further our understanding of the function of unc-53 we developed an in vivo assay to test gene fusions generated in vitro. Nematode expression vectors containing the full length unc-53 cDNA, TB3M5, downstream of various tissue specific and inducible promoters were constructed.

The mec-7 promoter of pTB112 (Fig. 7) confers tissue specific expression to the mechanosensory neurons, the unc-54 promoter of pTB111 (Fig. 7) confers tissue specific expression to body wall muscle and the hsp16-41 promoter of pTB109 (Fig. 7) confers and pTB110 (Fig. 7) confers heat inducible expression to somatic cells. pTB109 is a transcriptional fusion containing only the hsp16-41 gene promoter and has been shown to confer high levels of inducible expression in embryos. pTB110 contains a larger

portion of the hsp16-41/2 intergenic region in addition to a synthetic intron. This plasmid is expected to be highly inducible in embryos and post-embryonic stages in most somatic cell types.

5 Oocytes of both wild type (N2) and unc-53(n152) hermaphrodites were microinjected according to the method of Fire (1986), EMBO J., 5, 2673-2680. Coinjection of the unc-53 fusion with a selection  
10 plasmid, pRF4, a dominant marker of rol-6, allowed identification of transgenic animals by their right rolling phenotype (Mello et al, (1991), EMBO J., 10, 3959-3970. In C. elegans, the injected DNA does not integrate into the genome but rather forms  
15 extrachromosomal arrays which are heritable at a frequency ranging from 20-95% (Stinchcomb et al, (1985), Mol. Cell. Biol., 5, 3483-3496; Fire et al, (1990), Gene, 93, 189-198; Mello et al, (1991), EMBO J., 10, 3959-3970. Transgenic extrachromosomal lines were considered stable after the rolling phenotype had  
20 passed through four generations. Some transgenic HS-unc-53 strains were mutagenised with 3550 rads of  $\gamma$  rays emanating from a  $^{60}\text{Co}$  source which produces breaks in the chromosomes allowing for insertion of the extrachromosomal array. Stable integrants were  
25 identified by screening for homozygous rolling F3 broods. The names and genotypes of all transgenic strains are listed in Table 1 with details of the unc-53 fusions (constructs/vectors) listed in Table 2:

30 Table 1 - Extend in other constructs

STRAIN NAME	PARENTAL STRAIN	unc53 FUSION	SELECTION	lacZ MARKER
TB3In54	n152	pTB109	pRF4	UL6
35 TBAln8	N2	pTB110	pRF4	pPCZ1

5	TBAIn61	N2	pTB110	pRF4	pPCZ1
	TBAIn69	N2	pTB110	pRF4	pPCZ1
	TBAIn76 Accession No 1385CB (See Fig 17A)	N2	pTB110	pRF4	pPCZ1
	TBAIn90	N2	pTB110	pRF4	pPCZ1
	TBAIn210	N2	pTB110	pRF4	pPCZ1
10	TBAIn222	N2	pTB110	pRF4	pPCZ1
	TBAIn306	N2	pTB110	pRF4	pPCZ1
	TBAIn327	N2	pTB110	pRF4	pPCZ1
	TBBIn3	N2	pTB110	pRF4	pPCZ1
	TBBIn267	N2	pTB110	pRF4	pPCZ1
15	TB1Ex10	n152	pTB112	pRF4	none
	TB1Ex23	n152	pTB112	pRF4	none
	TB1Ex8	N2	pTB112	pRF4	none
	TB1Ex16	N2	pTB112	pRF4	none
	TB2Ex1	N2	pTB112	pRF4	none
20	TB2Ex37	N2	pTB112	pRF4	none
	TB3Ex10	N2	pTB112	pRF4	none
	TB3Ex12	N2	pTB112	pRF4	none
	TB3Ex20	N2	pTB112	pRF4	none
	TB3Ex37	N2	pTB112	pRF4	none
25	TB4Ex14	N2	pTB112	pRF4	none
	TB4Ex18	N2	pTB112	pRF4	none
	TB4Ex22	N2	pTB112	pRF4	none
	TB4Ex25 Accession No LMBP 1384CB (See Fig 16)	N2	pTB112	pRF4	none
	TB1Ex3	n152	pTB111	pRF4	none

TB1Ex6 (S e Fig 17B, C)	n152	pTB111	pRF4	none
TB1Ex11	n152	pTB111	pRF4	none

5

Notes for Table 1:

Ex-extrachromosomal

In-integrated

pTB109, pTB110-Heat shock unc-53 fusions

10 pTB111-mec-7 fusion

pTB112-unc-54 fusion

pRF4-rol-6 (sul006) (Mello et al, (1991), EMBO J., 5,  
3959-3970)

UL6-excretory canal promoter lacZ fusion

15 pPCZ1-Hsp16-48/1 lacZ fusion (Stringham et al, (1992)Molec.Biol.Cell 3, 221-233)Table 220 Full length cDNA tb3M5 (still has SL1 and 5' UTR)pTB50 (NotI-ApaI fragment in Bluescript II-KS, for  
in vitro transcription)pTB51 (NotI-ApaI fragment in Bluescript II-SK, for  
in vitro transcription)25 pTB54 (NotI-ApaI fragment in pCDNA3, for mammalian  
expression)

(Deposited as accession no. LMBP3296)

pTB109 (NotI-ApaI fragment in hsp16-pucBM21, for in  
vivo expression)

30 pTB67 (NotI-Apa fragment in pGEM5 +)

PCR1 of amino terminus of cDNA

(\*PCR using oligos BG01 and BG02)

pTB57 (NdeI-BamHI fragment in pRK172, for E. coli  
expression)

35

pTB58 (NdeI-NcoI fragment in pGEM5)

pTB63 (SacI-NcoI fragment in pRSETA, for E. coli  
expression)

pTB64 (BamHI fragment in pBluescriptII-KS)

5 Full length cDNA utilizing PCR1 amino terminus

pTB61 (NdeI-NcoI fragment in pRK172, for E. coli  
expression)

pTB110 (XbaI-KpnI fragment in pPD49.83, for in vivo  
expression)

10 pTB111 (XbaI-KpnI fragment in pPD52.102, for in  
vivo expression)

pTB112 (XbaI-KpnI fragment in pPD30.38, for in vivo  
expression)

(Deposited as accession no. LMBP3295)

15

PCR2 of amino terminus of cDNA

(\*PCR using oligos BG03 and BG01)

pTB59 (NotI-BamHI fragment in pBluescript II-KS)

20 pTB60 (NotI-XhoI fragment in pCDNA3, for mammalian  
expression)

Full length cDNA utilizing PCR2 amino terminus

pTB55 (NotI-EaeI fragment in pBluescriptII-KS)

25 pTB56 (NotI-ApaI fragment in pCDNA3, for mammalian  
expression)

Other constructs

pTB52 (SacII deletion of amino terminus of pTB50)

pTB53 (SacII deletion of amino terminus of pTB51)

30 pTB62 (SmaI fragment of pTB52 in pGEX2T, for  
prokaryotic expression)

pTB65 (NdeI-NcoI fragment of 3' terminus in  
pRK172, for prokaryotic expression)

35 pTB66 (NdeI-EcoRI fragment of 3' terminus in  
pRK172, for prokaryotic expression, MAB 16-  
48-2)



Initially, the phenotype of each transgenic line was characterised by inspection with a dissecting microscope and/or Nomarski optics. Transgenic strains were directly analysed for expression of unc-53 by immunohistochemistry. Briefly, embryos were adhered to polylysine coated slides and permeabilised by a combination of freeze fracturing and immersion in cold methanol and acetone (3-4 minutes each). Embryos were rehydrated through an acetone/distilled water series and then incubated for 30 minutes at room temperature in TBS-Tween (0.1%). The anti-UNC-53 monoclonal 16-48-2 anti-sera was applied undiluted and the slides incubated at 4°C overnight. The embryos were washed three times with TBS-T and then incubated in a secondary rhodamine like (Cy3-M) conjugated antibody for 1 hour at 37°C. After 3-4 washed in TBS-T the slides were mounted for fluorescence microscopy in 2% propylgallate, 80% glycerol-pH 8.0.

20 Characterisation of transgenic strains carrying pTB112

UNC-53 was over-expressed in the muscle of wild type animals (pTB112 in N2). Each extrachromosomal pTB112/N2 line consisted of wild type and rolling animals as expected, but in addition, several mutant phenotypes were observed at low frequency. These animals varied considerably in phenotype and included embryos which arrested at the two fold stage, larvae which hatched but died soon afterward, animals with extra protrusions on the epidermis and animals with a truncated posterior end. This phenotype is consistent with that of the mup or mua classes of muscle mutants in which the positioning and/or integrity of muscle attachments to the hypodermis has been disrupted. Most of these animals were either inviable or sterile. The progeny of the viable mutants contained the same

frequency of rollers, wild type and mutants as did the progeny of rolling individuals. Since the extrachromosomal array may be lost at each cell division, every animal is a mosaic. The healthy rollers may have lost the transgene from most muscle cells and may represent weak phenotypes whereas the 2 fold arrests represent the situation where the array has been lost from few muscle cells. Nomarski and polarised light microscopy of the severe larval lethals showed that the muscle cells were disorganised and over-extended.

Detailed analysis of the underlying defect in embryonic development that leads to this terminal phenotype were performed with immunofluorescence microscopy (Fig 21).

Since the *unc-54* gene encodes the myosin heavy chain, we expected that this promoter would be active in body muscle descendants from the comma stage onwards. In the *unc-54 - unc-53* strains, signal was indeed localised to the body muscle cells in comma and later stages as predicted. The immunofluorescence was localised to the cytoplasm of the cell bodies and was particularly intense at the tips of the extending processes. Increased process length was observed very early in muscle development (comma to 1.5 fold stage) and increased up to the three fold stage. No other abnormalities in shape or muscle myofilament pattern were observed in the anterior-posterior axis of the animal. Two and three fold embryos which were stained with the monoclonal antibody NE8(4c6.3) (Goh and Bogaert, (1991), Dev. Biol. 56, 110-156) appeared to have a relatively wild type myofilament structure. As these animals are mosaic, it may be possible that severe cases die in late morphogenesis and those which survive through embryogenesis to adulthood can tolerate a few distorted muscle cells.

pTB111 transgenic lines

Immunostains indicates that the transgene is expressed efficiently in the mechanosensory neurons of a transgenic extrachromosomal line carrying the pTB111 transgene in an unc-53 (n152) genetic background (Fig 20).

pTB109 and pTB110 lines

10

Twelve integrated lines derived from three separate mutageneses of extrachromosomal lines have been isolated. TB3In54 carries the pTB109 fusion in addition to pRF4. Nine TBA strains were isolated after mutagenesis of an extrachromosomal strain, HSA. There are two strains (TBB) derived from mutagenesis of the extrachromosomal strain HS B. Both TBA and TBB strains contain the transgenes pTB110, pPCZ1 and pRF4. Inclusion of the HS-lacZ plasmid, pPCZ1 (Stringham et al, (1992), Molec.Bio.Cell 3, 221-233) allows one to monitor the strength of the heat shock induction by assaying for  $\beta$ -galactosidase activity.

Immunostains of embryos freeze fractured after a two hour heat shock showed that the signal was most prominent in the pharynx, gut and neurons. Surprisingly, the signal had a speckled appearance. This may be a feature of heat shock. Heat shock proteins may sequester UNC-53 to "chaperone" it during stress. Alternatively, UNC-53 may be targeted for degradation. In one experiment, embryos were heat shocked for two hours, allowed to recover overnight and then freeze fractured the next morning. While levels were reduced, there was a little residual UNC-53 signal in the gut cells. Thus, about 16 hours later most the protein has gone.

Level of heat shock and recovery times are

therefor important factors in the mutant rescue experiments and the preferred assay system described in example 10. In addition, experiments suggest that heat shock induction in liquid culture versus agar plates or dry incubators versus water baths need careful calibration.

After a strong three hour heat shock, a high percentage of animals were not able to recover from the stress. Embryos which were not subjected to a double shock (2-two hour heat shocks at 33°C separated by a two-hour recovery) hatch out as malformed worms reminiscent of the muscle overexpression lines (Fig 21). The heat shock promoter used is especially active in the pharynx. Consistent with this, a strong pharyngeal morphogenetic phenotype was observed (Fig 21). Pharyngeal phenotypes are easy to score and quantify (feeding rate, dye uptake, LacZ lines staining the pharynx) by anyone skilled in the C. elegans field and may form a preferred embodiment of the assay.

#### Example 7

Over-expression of UNC-53 results in directional over-extension : Assay with 7A variant.

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In wild type *C. elegans*, body muscle cells are normally spindle shaped while in UNC53 mutants, a number of these cells have a reduced process which results in a fork shaped tip. This phenotype is consistent with the general reduction of extension observed in many growth cone types along the longitudinal axis of the animal in unc-53 mutants. Recalling the extremely limited pattern of UNC53 expression in embryogenesis detected by immunostaining with monoclonal antibody 16-48-2;no UNC53 activity was

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discernable in wild type body muscle cells during outgrowth suggesting that the levels of UNC53 activity required for this extension may be extremely low.

We overexpressed unc-53 in the muscle of wild type animals by expressing the full length cDNA under the control of the unc-54 myosin heavy chain promoter in the fusion pTB113. Plasmid pTB113 is a translational fusion containing the 7A variant unc-53 cDNA sequence as an XbaI-KpnI fragment starting from the first methionine and including the unc-53 cDNA poly adenylation tail under control of the myosin heavy chain unc-54 promoter of the nematode expression vector pPD30.38 available on Internet web site ftp archive: ciwl, ciwemb.edu. Plasmid pTB114 contains the identical cDNA fragment under control of the hsp16-41 -2 promoter (Jones et al., 1995, Dev. Biol. VOL. 171, PAGES 60-72) which confers heat inducible expression to somatic cells, in the expression vector pPD 49.83 (Fire, pers. comm.) The amino terminus of the UNC53 cDNA is identical to the PCR amplification with BG01 and BG02 of pTB57. Thus, both pTB113 and pTB114 are in frame translational fusions devoid of the SL1 leader sequence and upstream untranslated region of the cDNA.

Each transgenic mosaic line (3 were examined) consisted of wild type and rolling animals as expected, but in addition, several mutant phenotypes were observed at a low frequency. These animals varied considerably in phenotype and included, embryos which arrested at the two fold stage, larvae which hatched but died soon afterwards, animals with extra protrusions on the epidermis and animals with a truncated posterior end. Most of these latter animals

were either inviable or sterile. The progeny of the viable mutants contained the same frequency of rollers, wild type and mutants as did the progeny of rolling individuals. Since the extrachromosomal array may be lost at each cell division, every animal is a mosaic. The healthy rollers may have lost the transgene from most muscle cells and may represent weak phenotypes whereas the 2 fold arrests represent the situation where the array has been retained in most muscle cells. The truncated posterior end may be the result of lethality in the D lineage due to mosaicism. Nomarski and polarized light microscopy of the severe larval lethals showed that the muscle cells were disorganized and over-extended in the longitudinal axis. In some cases the muscle cells appeared detached from the hypodermis. As these animals are mosaic, it may be possible that severe cases die early in morphogenesis whereas those which survive through embryogenesis to adulthood can tolerate a few distorted muscle cells.

In transgenic pTB113 strains, UNC53 expression, as detected by immunostaining with monoclonal antibody 16-48-2, was localized to the body muscle cells in comma and later stages as predicted for the UNC-53 promoter (myosin heavy chain). The pattern of immunofluoresence with the anti UNC-53 antibody was localized to the cytoplasm of the cell bodies and was particularly intense at the tips of the extending processes and in the cytoskeleton, when compared to phalloidin staining which specifically stains the actin cytoskeleton. The identical pattern of sub-cellular localization, in the cytoplasm and cytoskeleton, was also observed in the intestinal

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cells of pTB114 transgenic embryos expressing UNC-53 ectopically after heat shock.

In addition, the growth cone processes appeared to be overextended specifically in the anterior-posterior axis of the animal. To verify this, the length of body muscle cells over-expressing the UNC53 cDNA in the pTB113 strains were measured and compared to the length of wild-type muscle growth cones expressing an unc-54 promoter-GFP (green fluorescent protein) fusion, pPD49.83 (available on Internet Web Ste Ftp archive: ciwl. ciwemb.edu. The GFP reporter allowed visualization of the entire cell body and boundaries of the muscle cells in wild-type animals. We estimated that the processes of the pTB113 expressing cells were roughly 1½ times the length of pPD49.83 expressing wild type cells.

The lethality in the transgenic progeny of the three pTB113 strains examined ranged from 32% to 78%. Thus a significant proportion of the transformed mosaic progeny did not survive morphogenesis. In contrast, no lethality was observed in the pPD93.48 (unc-54-GFP) control strains. The lethality observed in the pTB113 lines is likely the consequence of overextension of muscle cells during embryogenesis because (a) both pTB113 and pPD93.48 utilize the identical promoter and should be expressed in the same cells at the same point in development, and (b) rol-6 selection was used to identify transformants for both constructs.

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#### Example 8

Transient and stable transfection of UNC-53 in N4 neuroblastoma cells.

pTB72 and a plasmid expressing LacZ under the CMV promoter were transfected transiently with the Calcium-phosphate method in N4 neuroblastoma cells.

N4 cells and their stably transfected counterparts were grown in Minimum Essential Medium (MEM)-REGA 3 (GIBCO BRL) supplemented with 10% Foetal Calf Serum, 1% L-Glutamine, 2% Sodium Bicarbonate, 200 units/ml penicilline and 200 µg/ml Streptomycine, in a humidified atmosphere of 90% air and 10% CO<sub>2</sub> at 37°C.

Transfections were performed by the Lipofectamine method (GIBCO BRL). 18 to 24 hrs before transfection cells were seeded in complete growth medium at a density of 7x10<sup>5</sup> per well in a six well tissue culture plate, and incubated at 37°C in a CO<sub>2</sub> incubator. For each transfection the following solutions were prepared.:

SolA = 4 µg of DNA diluted in 200 µl of Optimem (GIBCO BRL)

SolB = 12 µl of Lipofectamine reagent diluted in 200 µl of Optimem (GIBCO BRL)

Solutions A and B were combined, gently mixed and incubated at room temperature for 30 minutes. For each transfection 0.6 ml of Optimem was added to the lipid-DNA complex to reach the final volume of 1 ml.

This mixture was then added onto the cells (which had been previously rinsed once with 2 ml of Optimem). The cells were incubated in the transfection mixture for 5 hrs at 37°C in a CO<sub>2</sub> incubator. At the beginning of the sixth hour from transfection, 1 ml of complete growth medium supplemented with 20% of Foetal calf serum was added to the transfected cells. The cells were incubated for 18 hrs at 37°C in a CO<sub>2</sub> incubator. 24 hrs following the beginning of transfection the supernatants was replaced with fresh growth medium.

72hrs post transfection cell cultures from each well were harvested, diluted 1:24 and distributed over 24



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well plates with the growth medium containing 500, 750 ug/ml or 1mg/ml of geneticin (G418, GIBCO BRL). After ~12 days from the start of selection, single clones were picked and allowed to grow in the absence of selection. Of 27 initial clones, 7 were lost while expanding the clones because of their slow growth rate and the apparent general toxicity of caused by the transfected construct. Clone 9 was selected for further analysis.

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Functional assay for neurite extension in N4 neuroblastoma

Step (1): Quantitative determination of neuronal morphology, i.e. length of neurites and fraction of positive cells is performed fully automatically. As an example we studied the degree of morphological differentiation in the wild-type N4 cells to a stably transfected C9 clone.

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Step (2): Quantitative neuronal morphology

Morphological changes of neurones were quantitated as described in GEERTS et al (1992 Restorative Neurology and Neuroscience 4: 21-32 and Katsuhito et al Neurodegeneration, 2: 173-181). Briefly, at appropriate times, glutaraldehyde was applied to cell cultures. No washing steps were performed. This ensured that the morphology of the cells at that time point was frozen. The cells were observed in transmitted light mode on an Axiovert microscope, equipped with a Marzhauser scanning stage driven by an Indy workstation (Silicon graphics). Images were captured using a MC5 video camera (HCS). About 3000 cells were detected in 64 neatly aligned images, forming a 8x8 square matrix of images. The exact alignment of the images ensured that neurites

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could be followed from one image field to the next. The analysis software automatically detected cell bodies and neurites and saved cell body size and length of each individual neurite on a file.

5 Different parameters were subsequently calculated. The neurite length per cell was calculated on freely lying cells (not within a cluster). The fraction positive cells is the fraction of cells having at least one neurite with a length exceeding twice the  
10 cell body diameter. Figure 40 clearly shows that clone C9 increases both neurite length (free length) and fraction of positive cells, compared to wild-type N4 cells clone.

15           Example 9

Transient and stable transfection of UNC-53 in MCF-7 breast carcinoma cells.

pTB72 and a plasmid expressing Lac Z under the CMV promoter were transfected transiently with the  
20 Ca-phosphate method in MCF-7 breast carcinoma cells.

MCF7 cells and their stably transfected counterparts were grown in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO BRL) supplemented with 10% foetal Calf Serum, 1% L-Glutamine, 1% of a 5mg/ml stock of  
25 Gentamicine and 1% of a 100mM stock of Sodium Pyruvate in an humidified atmosphere of 90% air and 10% CO<sub>2</sub> at 37 C. Construct pTB72 was transfected by the Calcium-phosphate method (ref): 18-24hrs before transfection. cells were seeded at a density of  $3 \times 10^5$  in a six well  
30 tissue culture plate with complete growth medium. Two hours before transfection the culture medium was removed and replaced with 1.8 ml of fresh medium. The cells were put back in the incubator until the moment of transfection. DNA-Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> precipitates were  
35 prepared one hour before transfection : For each transfection (1 well): 4 ug of DNA (=3-4 ul) was

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combined with 76 ul of TE (Tris HCl-EDTA pH 8) 0.1M to a final volume of 80 ul. To these DNA's diluted in TE, 20 ul of CaCl<sub>2</sub> Hepes solution was added to a final volume of 100 ul of DNA/CaCl<sub>2</sub> mixture. The 100 ul of DNA/CaCl<sub>2</sub> mixture was added very slowly, drop-by-drop to 100ul of 2x BS/Hepes while shaking, to a final volume of 200 ul. The resulting 200 ul DNA/Calcium Phosphate mixture was added to the cells and the mixture incubated for 8 hrs at 37 C in a CO<sub>2</sub> incubator. At the beginning of the ninth hour from the start of transfection, the supernatans with the DNA/Calcium phosphate mixture was replaced with 3 ml of complete culture medium. 72hrs post transfection, cells from each well were harvested, split 1:24 in complete growth medium supplemented with 1mg/ml of Geneticin (G418, GIBCO-BRL) and plated out in 24 well plates. 15 days from the start of selection, single clones where picked and allowed to grow without selection. Three clones MCF7-pTB72-clone9, MCF7-pTB72-14 and MCF7-pTB72-15 were retained all of which have a similar phenotype.

1) Phenotyping UNC-53 transfected MCF-7 breast carcinoma cells:

The general morphology and motile behaviour of the three transfected MCF-7 clones are different from non-transfected cells.

The assay consists of a tyramide amplification of a classical immunofluorescent reaction. The cells were grown in defined medium with 10% charcoal treated serum and supplemented by 10 µg/ml insulin (final concentration) and 5 ng/ml basic fibroblast growth factor (final concentration). The substrate consisted of 50 µg/ml poly-L-lysine in chamber slides; cultures were maintained in a humidified atmosphere of 95/5% air/CO<sub>2</sub>.

Induction of expression of vimentin and of increased levels of fosfotyrosine was found in the transfected subclones. Vimentin formed dense clusters around the cell nucleus with some filamentous structures in the pseudo-podes. Fosfotyrosine, on the other hand, was predominantly found at the border of the cell ruffles, at the same subcellular area where UNC53 expression was found. This provides evidence of a controlling molecule functioning in a signal transduction pathway and that vimentin is an indicator of metastasis in cancerous cell lines.

2) Functional assay to establish the signal transduction role of UNC-53.

Cells locomote in tissues and on substrates. The type and amount of cell locomotion depends on different factors: (1) the physiological conditions perceived through receptors, which can be - for example - stimulation with or deprivation of serum, growth factor(s), cytokine(s), chemokine(s) or (pro-) inflammatory mediators; (2) the type and functionality of cell adhesion molecules expressed by cells and extracellular matrix molecules present in tissue or in culture model, (3) the actin, tubulin and/or intermediate filament cytoskeleton and (4) proper functioning of integrator proteins such as UNC-53, homologues or other molecules that translate physiological stimuli (or lack of stimuli) into increased or decreased cell motility, directional or random motility or different types of motility. Cell locomotion can be measured in different types of assays, such as disperse cells or in monolayer cultures, as cellular outgrowth from tissues in culture or in organotype cultures. Motility of live cells can be quantified microscopically as in example 8 or by time-lapse video or cinematography or by

phagokinetic assays (Albrecht-Buehler, 1977, Cell, 11:395) amongst other methods.

Cell motility assays are interesting tools to study the functioning and pharmacology of UNC-53 and the unc-53 pathway.

All previous observations were performed on MCF-7 cells grown in defined medium supplemented by 10 µg/ml insulin (final concentration) and 5ng/ml basic fibroblast growth factor (final concentration). This approach offers the possibility of investigating the role of FGF in the UNC53 role of signal transmission. Indeed, by comparing wild-type versus UNC53 transfected cells cultured in medium with or without FGF/insulin and/or by microinjection of UNC53 protein, it can be investigated if UNC53 is responsible directly for regulating a signal transduction pathway linking extracellular growth factors to the assembly of, amongst others, focal adhesions.

Example 10: Enhanced phagokinesis in Ce-unc-53 transfected MCF-7 cells.

In this example evidence is presented that transfection of a plasmid containing the Ce-unc-53 sequence under a suitable promoter enhances cell motility in the phagokinesis assay.

When culture plastics are coated with colloidal gold particles, a variety of cells types were shown to migrate over the plate and displace or phagocytose the gold lawn on their way while locomoting. The track left bare is a qualitative and quantitative measure of cell motility and/or locomotion. The basic methods have been described in detail elsewhere (Albrecht-Buehler, 1977, Cell, 11:395; Zetter, 1980, Nature, 285:41; O'Keefe et al., 1983, J. Invest. Dermatol., 85:130).

### Methods

12 well plates were coated for 15 minutes with 5  $\mu$ g/ml gelatin in water and gold coated as described by Albrecht-Bueller (1977). Ce-unc-53 transfected MCF-7 cells and the parent MCF-7 were cultured in parallel, trypsinised dispersed in culture medium and seeded in 12-well plates at a density of 2550 cells per well. The cells were allowed to adhere to the plate and to locomote for 16 hours. After incubation the cells were chemically fixed to the plate using paraformaldehyde, washed with distilled water and finally air-dried.

Subsequently, images of the gold lawns were captured using automated videomicroscopy, composite images of the wells were generated and single-cell phagokinetic tracks were measured using a home-made routine in SCIL<sup>TM</sup> software.

### Results

The parent MCF-7 line displayed two cell populations with different motile behaviour in phagokinesis assays. In table 3 the fraction of parent and Ce-unc-53 transfected MCF-7 cells that produced linear tracks in the phagokinesis assay are shown. In the parent MCF-7 cells, 88% of the cells produce a round track (long and short axis less than 2-fold different) and 12% cells produce 'linear' tracks (long and short axis more than 2-fold different). Ce-unc-53 transfection of MCF-7 cells produced an increase of the fraction of cells displaying 'linear' tracks to 28% at the cost of the cells producing round tracks.

These observation suggest that Ce-unc-53 transfection into MCF-7 is capable of increasing *in situ* locomotion of MCF-7 e.g. by increased spreading, ruffling or other forms of non-directional motility in

the 'round' population as well as by driving a fraction of transfected MCF-7 cells from non-directional motility (round tracks) into directional migration (linear tracks).

5           In tissue culture, cells are provided with non-directional signals. It is likely that providing directionality to these signals will enhance observed effects. Significant enhancement was observed for the fraction of linear tracks.

10           In addition, a significant increase of 35% in the area of tracks was observed in the Ce-unc-53 transfected MCF-7 cells versus the parent MCF-7 cells (Table 3). This increase occurred in the round track population; the area of linear tracks was found not to  
15 be changed by transfection.

          These observations in phagokinesis suggest that Ce-unc-53 transfection into MCF-7 cells is capable of increasing insitu locomotion in Ce-unc-53 MCF-7, e.g. by increasing spreading, ruffling, or other forms of  
20 non-directional motility in the "round" population. In addition the Ce-unc-53 transgene in MCF-7 cells drives a fraction of the MCF-7 cells from non-directional motility (round tracks) into directional migration (linear tracks).

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**Table 3. Analysis of phagokinesis assays with parent and Ce-unc-53 transfected MCF-7 cells.**

	parent MCF-7		Ce-unc-53 MCF-7		increase
Fraction linear tracks (*)	% +- SD(n) 12+-3 (8)		%+-SD(n) 28+-6 (8)		2.33
Track area (**)	pixels+-SD(n)		picels+-SD (n)		
all tracks	1261+-128(8)		1698+-179(8)		1.35
round tracks	1229+-162(8)		1464+-204(8)		1.19
linear tracks	2367+-424(8)		2300+-319(8)		0.97

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(\*) the fraction of linear tracks in 8 wells was pooled.

5 MCF-7 cells expressing low levels of UNC-53  
exhibit increased motility.

Individual transfected cells are much more  
flattened in appearance than wild type and have a  
broad lamellipodium extending from the edge of the  
cell. Ruffling edges are more frequent than in wild  
10 type. Transfected cells in clusters have a broad  
lamellipodium edge around the cluster while cluster of  
the non-transfected. Within the cluster the nuclei are  
more widely spaced from one-another than in wild type  
cells (also due to a lamellipodium edge).

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#### Example 11

Method for Protein micro-sequencing of co-  
affinity purifying proteins

20 UNC-53 protein was immuno-affinity purified from  
extracts of cells expressing C. elegans UNC-53 using  
monoclonal antibody 16-48-2. One to five mg of Mab  
16-48-2 was prepared, purified on protein-G sepharose  
and subsequently covalently linked to sepharose beads.  
A column of such beads was loaded with both crude  
25 cytosolic and Triton-X100 extracts (containing  
solubilised RTKs) and eluted with 4M MgCl<sub>2</sub> or other  
chaotropic agents. A co-immuno-purifying band was  
identified on SDS-denaturing PAGE gels, eluted from  
these gels and micro-sequenced. This protein sequence  
30 or mass information of peptides generated by  
proteolysis was used to identify the co-  
immunoprecipitation directly from the sequence  
databases.

Alternatively the sequence was reverse translated



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and oligonucleotides based on the sequence prepared. This is used to clone the corresponding gene as well as other techniques well known in the art.

5           Example 12   C. elegans as a model assay system.

We have constructed transgenic strains which overexpress UNC-53 in body muscle. This results in increased extension of muscle cells and embryonic lethality at low frequency. These strains were used  
10 to screen for drugs which interfere with UNC-53 activity and thereby suppress the background lethality.

Another related assay was used to screen specifically to identify inhibitors of downstream  
15 components in the signal transduction pathway. This assay utilised constitutively active mutant cDNA (or corresponding nucleic acid sequence). Such a mutant may be formed by mutating the nucleotide binding domain such that GTP or ATP is always bound or by  
20 covalently attaching SEM-5. In this strategy, transgenics/mutants (nematodes or tissue cultured cell lines) were generated which maintain the pathway in a permanently switched on state. Over-extension and subsequent lethality results in a greater frequency  
25 than that observed in the unc-54 - unc-53 wild-type lines. By screening for survivors after drug treatment, this assay specifically identifies inhibitors of downstream components in the signal transduction pathway.

30           A range of other embodiments of the assay are obvious to a person skilled in the art of C. elegans genetics, including the use of alternative selectable markers, genetic backgrounds, histochemical detection and visual detection systems to identify phenotypic

changes following contacting a single worm or a population of worms with a compound.

Another assay previously described herein utilizes the *unc-53* promoter. The *unc-53* promoter is fused to a nucleic acid sequence encoding a reporter molecule. By screening for cells which do not express the wild type pattern, molecules which increase or reduce transcription of *unc-53* may be identified.

10        Example 13 - Heterologous expression of  
*C. elegans* UNC-53 in insect cells.

*C. elegans* UNC53 cDNAs have been expressed in a Baculovirus system to obtain sufficient amounts of protein for biochemical and structural studies.

15        Two UNC53 cDNA clones (UNC53(7A) and UNC53(8A)) have been documented differing in the number of adenosine (A) residues (7 or 8) in a polyA stretch of the of the 3' coding region; the two clones therefore have different reading frames in the carboxyterminal  
20        coding region.

The 5' (N-terminal) part of the UNC53 coding region was excised from pTB564 with *SacII* after linearizing the plasmid with *NdeI*. The *NdeI* site was blunted with Klenow. The remaining C-terminal part of  
25        the coding region was excised from pTB68(7A) and pTB50(8A) with *SacII* plus *KpnI*. The *NdeI/SacII* fragment from pTB64 and the *SacII/KpnI* fragment from either pTB68 or pTB50 were ligated simultaneously into pBacPAK9 (Clontech) which had been linearized with  
30        *Ecl136II* (blunt end) and *KpnI*. In this way, a minimum amount of 5' untranslated region is left in the final construct.

The desired recombinant viruses were obtained by

co-transfection of Sf21 cells (*Spodoptera frugiperda*) with one of the aforementioned pBacPAK9 constructs and BacPAK6 Bsu361-digested DNA (Clontech). Several candidate recombinant viruses plaques were picked and  
5 screened by PCR for the presence of the target gene and the absence of wild-type virus.

Sf9 cells were infected at a high multiplicity with UNC53(7A) or UNC53(8A) recombinant Baculoviruses for protein expression. Proteins from whole cell  
10 lysates were separated by denaturing (SDS) polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The expression of UNC53 in those cell lysates was confirmed by immunoreaction with a monoclonal antibody (16-49-2) to UNC53 and  
15 subsequent chemiluminescent detection (ECL™ Amersham). A Coomassie-stained band of the expected size was observed in lysates of Sf9 cells infected with UNC-53(7A) or UNC53(8A) recombinant baculoviruses, but not with control constructs.  
20 Within the accuracy of the methods, this Coomassie-stained band coincided with the largest immunoreactive band. Their estimated mass was approximately 180 kDa, which is compatible with the theoretically calculated mass (167 kDa). We therefore conclude that this band  
25 most likely corresponds to intact UNC53.

For both UNC53(7A) and UNC53(8A) baculoviral expression constructs, mostly intact recombinant UNC53-protein was detected by immunoblotting in  
lysates from infected cells harvested 24 hours post  
30 infection. Larger amounts of recombinant protein could be detected in lysates from cells prepared during later stages of infection (48 and 72 hours post infection) but in those preparations a considerable amount of smaller fragments (presumptive degradation  
35 products) is observed.

Example 14

The UNC-53 protein expressed in Sf9 cells using a Baculovirus expression system is a valid tool to study its biochemical functions and a valid tool to identify interacting proteins.

3x10+6 SF9 cells infected with recombinant virus UNC53 7A(L2.3)/pBacPAK9 were resuspended in 100 microliter Phosphate Buffered Saline supplemented with 0.14 micromolar of pepstatin, 10 mM of benzamidine and 0.015 micromolar aprotinin. The cells were briefly sonicated and the obtained material was centrifuged at 30,000 g for 30 minutes at 4 degrees centrigade. The clear supernatant (soluble fraction) was frozen in 50% glycerol. An aliquot of this fraction was incubated in the cold room for 48 hrs. The protein samples were analyzed by SDS-PAGE, blotted to nitrocellulose and probed with mab 16-48-2. This showed that UNC-53 protein made in SF9 cells is soluble and stable under the conditions tested.

20 microlitres of the UNC-53 SF9 lysate were incubated with 5 microlitre GST-Sepharose beads loaded with equal amouts (approx. 10 microgram) of GST-GRB-2 or GST alone. The beads were rinsed 3 times in 500 microlitres of solution PBS-0.2% Tween 20 and eluted with 50 microliter SDS sample buffer. The eluted material was analyzed by SDS-PAGE and Western blot analysis with mab 16-48-2. UNC-53 was retained on the GST-GRB2 column and not on the GST demonstrating that UNC-53 interacts in vitro with GRB-2.

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Example 15

Identification of proteins interacting with UNC-53 :

5            Vectors pCB50 and pCB51 were constructed as bait vectors for the yeast two hybrid system expressing resp. the full length and the carboxyterminal part of UNC-53.

10           pCB50 was constructed by cloning the full length UNC-53 cDNA (7A variant; NdeI-NcoI fragment from pTB74) into pAS1-CYH2 vector from Clontech. (Figure 30).

15           pCB51 (Figure 32) was constructed by cloning the 1880 bp NdeI-NcoI fragment from pTB74 into vector pAS1-CYH2 from Clontech. This protein encodes among others, the GTP/ATP binding domains, a leucine zipper domain, and an additional coiled-coil domain.

20           pCB50 and pCB51 were transformed in yeast strain Hf7C (YRG2). Expression was confirmed by western blotting using antibodies to the GAL4 protein fused to UNC-53 in these constructs. Bands of expected size (190 kd for pCB50 and 90 kd for pCB51) were observed both in yeast strains with pCB50 and pCB51 indicating that both fusion proteins are expressed in the yeast.

25           The expression of the pCB50 and pCB51 fusion proteins in yeast strain Hf7C does not lead to expression of the LacZ or HIS reporter genes. These experiments demonstrate that the constructed fusions are useful baits in yeast two hybrid screens.

30           Vector pCB55 was made by cloning the 984 bp BamHI-BglIII of pTB74 construct into the yeast two

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hybrid activation vector (pGAD-424 vector from Clontech) (Figure 34). In order to check the possible interactions of UNC-53 either with itself (homodimerization) or other proteins.

- 5           This vector expresses a Gal-4 activation domain fused to amongst others the predicted coiled coil or leucine zipper domain of UNC-53.

10           The following combinations of plasmids were co-transformed in yeast strain HF7C : (1) pCB51 and pCB55 (2) pCB55 with control plasmid- pTD1 and (3) positive control plasmids pTD1 and PVA3 (two proteins known to interact (Bartel, P.L et al., Biotechniques Vol. 14 nr.6 (1993))). Yeast cotransformed with combination (1) and (3) grew well on -LEU;-TRYP plates and -LEU;-TRYP;-HIS plates indicating that an interacting protein is present in both co-transformations. Only yeast co-transformed with (3) was positive in a lacZ assay indicating that the observed interaction in (1) (between pCB50 and pCB 55) is weak. For co-  
15           transformation (2), colonies grew on -LEU;-TRYP plates and as expected not on -LEU;-TRYP;-HIS plates. The positive control were thus positive whereas the negative controls were negative. We conclude that there is a weak but significant interaction between  
20           pCB51 and pCB55, which is strong enough to activate the HIS but not the lacZ reporter gene in this Hf7c strain.

#### Example 16

- 30           Protocol to screen for components which inhibit or enhance UNC-53 using C. elegans cell line pTBIn76

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Embryos from large liquid C. elegans cultures of line pTBIn76 (table 1) are collected by sucrose flotation of a bleached population (Goh and Bogaert (1991), Dev. Biol. 56, 110-156). Embryos are  
5 dispensed in 96 well microtiter plates with M9 medium and various concentrations of the compound to be tested. The embryos are allowed to hatch and are synchronised in the L1 stage by starvation. After a suitable exposure to the compound (by standard  
10 calibration) a standard quantity of E. coli (food) is dispersed in the 96 well plates, which starts C. elegans post-embryonic development. The microtiter plates are then placed in an incubator to induce heat shock and subsequently placed at 25°C to permit  
15 continued development. After 0 to 1 generations of C. elegans development wells are inspected to assess the degree of population growth inhibition. This inspection can consist of an optical density measurement to assess the amount of food consumed by  
20 the developing nematodes. Very little food is consumed when no test compound is present: most food is consumed if an UNC-53 inhibitor has blocked the lethal or subviable phenotype induced by the transgene. The inspection can also be a visual  
25 inspection of the number of healthy or subviable worms or a histochemical measurement of C. elegans viability or of the remainder of E. coli (food).

Example 17 - Protocol to screen for compounds  
30 which inhibit or enhance cell regulation or motility.

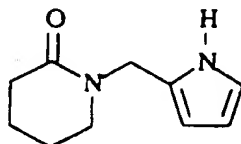
Transfected cells used in this example were the same as those obtained from example 8. Compounds to be tested were added to each of the cells and their  
35 effects on the cells monitored. Functional assays to determine neurite extension were also the same as used

in example 8 as described by Geests et al. One compound (of the Formula I below) was used for further testing.

5            Example 18 - Compounds targetted at the unc-53 pathway.

Synthesis of (1-(1H-pyrrol-2-ylmethyl)-2-piperidone.

10



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Step 1

To a stirred solution of 150g of 1H-pyrrol-2-carboxaldehyde in 1500g parts of trichloromethane were  
20       added 690, of 5Å molecular Sieves. A kit solution of 264, of methyl 5-aminopentanoate hydrochloride in 1500g of trichloromethane was added. After stirring for 5 minutes, 465g of thiethylamine were added over  
10 minutes. Upon complete addition, the reaction  
25       mixture was stirred for 20 hours at ambient temperature. The mixture was filtered over diatomaceous earth and the filtrate was concentrated by evaporation of the solvent. The concentrate was triturated in 1,1'-oxybisethane. The precipitate was  
30       filtered off and the filtrate was concentrated, yielding 300g (91.1%) of 5-[[1-(1H-pyrrol-2-



Step 2

A mixture of 150g of 5-[[(1H-pyrrol-2-yl)methylen]amino]pentanoate hydrogenated at  $3.10^5$  Pa and at ambient temperature with 3.3 parts of platinum oxide. After the calculated amount of hydrogen was consumed, the catalyst was filtered off and the filtrate was evaporated. The residue was dissolved in dichloromethane and the organic phase was washed three times with a sodium hydroxide 3 N solution. The product was distilled at 13.30 Pa (bp 100-130°C). The residue was crystallized from cyclohexane and hexane. The product was filtered off and dried, yielding 193 parts (100%) of 1-(1H-pyrrol-2-ylmethyl)-2-piperidone. ; mp. 105.8°C.

The compound (1-(1H-pyrrol-2-ylmethyl)-2-piperidinone) when applied for 24 hours to cultures of both wild-type and transfected N4 (mouse neuroblastoma) cells displays a differential behaviour. There is no effect (or at most a small stimulatory) effect on the wild-type N4 cells, up to concentrations of 1  $\mu$ M, the compound clearly becomes toxic for both types of cells. The results indicate that this compound counteracts the effects of overexpression of UNC-53 and may have beneficial effects therefore in for example metastasis.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

(A) NAME: BOGAERT; THIERRY  
(B) STREET: Voorstraat 36 bus 11  
(C) CITY: Kortrijk  
(E) COUNTRY: Belgium  
(F) POSTAL CODE (ZIP): B-8500

(A) NAME: STRINGHAM; EVE  
(B) STREET: 9326-133 A Street  
(C) CITY: Surrey  
(D) STATE: British Columbia  
(E) COUNTRY: Canada  
(F) POSTAL CODE (ZIP): V3V 5R5

(A) NAME: VANDEKERCKHOVE; JOEL  
(B) STREET: Rode Benkendreef 27  
(C) CITY: Loppem  
(D) STATE: -  
(E) COUNTRY: Belgium  
(F) POSTAL CODE (ZIP): none

(ii) TITLE OF INVENTION: Processes for the identification of compounds which control cell behaviour, the compounds identified and pharmaceutical compositions containing them and their use-- in the control of cell behaviour

(iii) NUMBER OF SEQUENCES: 48

## (iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

## (v) CURRENT APPLICATION DATA:

APPLICATION NUMBER: EP PCT/EP96/02311

## (vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: GB 9510944.3  
(B) FILING DATE: 31-MAY-1995

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5073 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: Caenorhabditis elegans

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GGTTTAATTA	CCCAAGTTTG	AGACATCAAT	TCCATCGAAC	GAAATGTTGG	TGCTCCGAAT	60
AAAATGACGA	CGTCAAATGT	AGAATTGATA	CCAATCTACA	CGGATTGGGC	CAATCGGCAC	120
CTTTCGAAGG	GCAGCTTATC	AAAGTCGATT	AGGGATATTT	CCAATGATTT	TCGCGACTAT	180
CGACTGGTTT	CTCAGCTTAT	TAATGTGATC	GTTCCGATCA	ACGAATTCTC	GCCTGCATTC	240
ACGAAACGTT	TGGCAAAAAT	CACATCGAAC	CTGGATGGCC	TCGAAACGTG	TCTCGACTAC	300
CTGAAAAATC	TGGGTCTCGA	CTGCTCGAAA	CTCACCAAAA	CCGATATCGA	CAGCGGAAAC	360
TTGGGTGCAG	TTCTCCAGCT	GCTCTTCCTG	CTCTCCACCT	ACAAGCAGAA	GCTTCGGCAA	420
CTGAAAAAAG	ATCAGAAGAA	ATTGGAGCAA	CTACCCACAT	CCATTATGCC	ACCCGCGGTT	480
TCTAAATTAC	CCTCGCCACG	TGTCGCCACG	TCAGCAACCG	CTTCAGCAAC	TAACCCAAAT	540
TCCAACTTTC	CACAAATGTC	AACATCCAGG	CTTCAGACTC	CACAGTCAAG	AATATCGAAA	600
ATTGATT CAT	CAAAGATTGG	TATCAAGCCA	AAGACGTCTG	GACTTAAACC	ACCCTCATCA	660
TCAACCACTT	CATCAAATAA	TACAAATTCA	TTCCGTCCGT	CGAGCCGTTT	GAGTGGCAAT	720
AATAATGTTG	GCTCGACGAT	ATCCACATCT	GCGAAGAGCT	TAGAATCATC	ATCAACGTAC	780
AGCTCTATTT	CGAATCTAAA	CCGACCTACC	TCCCAACTCC	AAAAACCTTC	TAGACCACAA	840
ACCCAGCTAG	TTCGTGTTGC	TACAACTACA	AAAATCGGAA	GCTCAAAGCT	AGCCGCTCCG	900
AAAGCCGTGA	GCACCCCAA	ACTTGCTTCT	GTGAAGACTA	TTGGAGCAAA	ACAAGAGCCC	960
GATAACAGCG	GTGGTGGTGG	TGGTGGAAATG	CTGAAATTAA	AGTTATT CAG	TAGCAAAAAC	1020
CCATCTTCCT	CATCGAATAG	CCCACAACCT	ACGAGAAAGG	CGGCGGCGGT	GCCTCAACAA	1080
CAAACTTTGT	CGAAAATCGC	TGCCCCAGTG	AAAAGTGGCC	TGAAGCCGCC	GACCAGTAAG	1140
CTGGGAAGTG	CCACGTCTAT	GTCGAAGCTT	TGTACGCCAA	AAGTTTCCTA	CCGTAAAACG	1200
GAGCCCCCAA	TCATATCTCA	ACAAGACTCG	AAACGATGCT	CAAAGAGCAG	TGAAGAAGAG	1260
TCCGGATACG	CTGGATTCAA	CAGCACGTCTG	CCAACGTCAT	CATCGACGGA	AGGTTCCCTA	1320
AGCATGCATT	CCACATCTTC	CAAGAGTTCA	ACGTCAGACG	AAAAGTCTCC	GTCATCAGAC	1380
GATCTTACTC	TTAACGCCTC	CATCGTGACA	GCTATCAGAC	AGCCGATAGC	CGCAACACCG	1440
GTTTCTCCAA	ATATTATCAA	CAAGCCTGTT	GAGGAAAAAC	CAACACTGGC	AGTGAAAGGA	1500
GTGAAAAGCA	CAGCGAAAAA	AGATCCACCT	CCAGCTGTTC	CGCCACGTGA	CACCCAGCCA	1560
ACAATCGGAG	TTGTTAGTCC	AATTATGGCA	CATAAGAAGT	TGACAAATGA	CCCCGTGATA	1620
TCTGAAAAAC	CAGAACCTGA	AAAGCTCCAA	TCAATGAGCA	TCGACACGAC	GGACGTTCCA	1680
CCGCTTCCAC	CTCTAAAATC	AGTTGTTCCA	CTTAAAAATGA	CTTCAATCCG	ACAACCACCA	1740
ACGTACGATG	TTCTTCTAAA	ACAAGGAAAA	ATCACATCGC	CTGTCAAGTC	GTTTGGATAT	1800
GAGCAGTCGT	CCGCGTCTGA	AGACTCCATT	GTGGCTCATG	CGTCGGCTCA	GGTGAATCCG	1860
CCGACAAAAA	CTTCTGGTAA	TCATTGCTG	GAGAGAAGGA	TGGGAAAGAA	TAAGACATCA	1920

GAATCCAGCG	GCTACACCTC	TGACGCCGGT	GTTGCGATGT	GCGCCAAAAT	GAGGGAGAAG	1980
CTGAAAGAAT	ACGATGACAT	GACTCGTCGA	GCACAGAACG	GCTATCCTGA	CAACTTCGAA	2040
GACAGTTCCT	CCTTGTCGTC	TGGAATATCC	GATAACAACG	AGCTCGACGA	CATATCCACG	2100
GACGATTTGT	CCGGAGTAGA	CATGGCAACA	GTCGCCTCCA	AACATAGCGA	CTATTCCCAC	2160
TTTGTTTCGCC	ATCCCACGTC	TTCTTCCTCA	AAGCCCCGAG	TCCCCAGTCG	GTCCTCCACA	2220
TCAGTCGATT	CTCGATCTCG	AGCAGAACAG	GAGAATGTGT	ACAAACTTCT	GTCCCAGTGC	2280
CGAACGAGCC	AACGTGGCGC	CGCTGCCACC	TCAACCTTCG	GACAACATTC	GCTAAGATCC	2340
CCGGGATACT	CATCCTATTC	TCCACACTTA	TCAGTGTGAG	CTGATAAGGA	CACAATGTCT	2400
ATGCACTCAC	AGACTAGTCG	ACGACCTTCT	TCACAAAAAC	CAAGCTATTC	AGGCCAATTT	2460
CATTCACTTG	ATCGTAAATG	CCACCTTCAA	GAGTTCACAT	CCACCGAGCA	CAGAATGGCG	2520
GCTCTCTTGA	GCCCGAGACG	GGTGCCGAAC	TCGATGTGCA	AATATGATTC	TTCAGGATCC	2580
TACTCGGCGC	GTTCCCGAGG	TGGAAGCTCT	ACTGGTATCT	ATGGAGAGAC	GTTCCAAGTG	2640
CACAGACTAT	CCGATGAAAA	ATCCCCCGCA	CATTCTGCCA	AAAGTGAGAT	GGGATCCCAA	2700
CTATCACTGG	CTAGCACGAC	AGCATATGGA	TCTCTCAATG	AGAAGTACGA	ACATGCTATT	2760
CGGGACATGG	CACGTGACTT	GGAGTGTTAC	AAGAACACTG	TCGACTCACT	AACCAAGAAA	2820
CAGGAGAACT	ATGGAGCATT	GTTTGATCTT	TTTGAGCAAA	AGCTTAGAAA	ACTCACTCAA	2880
CACATTGATC	GATCCAAGTT	GAAGCCTGAA	GAGGCAATAC	GATTCAGGCA	GGACATTGCT	2940
CATTTGAGGG	ATATTAGCAA	TCATCTTGCA	TCCAAGTCAG	CTCATGCTAA	CGAAGGCGCT	3000
GGTGAGCTTC	TTCGTCAACC	ATCTCTGGAA	TCAGTTGCAT	CCCATCGATC	ATCGATGTCA	3060
TCGTCGTGCA	AAAGCAGCAA	GCAGGAGAAG	ATCAGCTTGA	GCTCGTTTGG	CAAGAACAAG	3120
AAGAGCTGGA	TCCGCTCCTC	ACTCTCCAAG	TTCACCAAGA	AGAAGAACAA	GAAGTACGAC	3180
GAAGCACATA	TGCCATCAAT	TTCCGGATCT	CAAGGAACTC	TTGACAACAT	TGATGTGATT	3240
GAGTTGAAGC	AAGAGCTCAA	AGAACGCGAT	AGTGCACTTT	ACGAAGTCCG	CCTTGACAAT	3300
CTGGATCGTG	CCCGCGAAGT	TGATGTTCTG	AGGGAGACAG	TGAACAAGTT	GAAAACCGAG	3360
AACAAGCAAT	TAAAGAAAGA	AGTGGACAAA	CTCACCAACG	GTCCAGCCAC	TCGTGCTTCT	3420
TCCCGCGCCT	CAATTCCAGT	TATCTACGAC	GATGAGCATG	TCTATGATGC	AGCGTGTAGC	3480
AGTACATCAG	CTAGTCAATC	TTGAAACGA	TCCTCTGGCT	GCAACTCAAT	CAAGGTTACT	3540
GTAAACGTGG	ACATCGCTGG	AGAAATCAGT	TCGATCGTTA	ACCCGGACAA	AGAGATAATC	3600
GTAGGATATC	TTGCCATGTC	AACCAGTCAG	TCATGCTGGA	AAGACATTGA	TGTTTCTATT	3660
CTAGGACTAT	TTGAAGTCTA	CCTATCCAGA	ATTGATGTGG	AGCATCAACT	TGGAATCGAT	3720
GCTCGTGATT	CTATCCTTGG	CTATCAAATT	GGTGAAGTTC	GACGCGTCAT	TGGAGACTCC	3780
ACAACCATGA	TAACCAGCCA	TCCAAGTGAC	ATTCTTACTT	CCTCAACTAC	AATCCGAATG	3840

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TTCATGCACG GTGCCGCACA GAGTCGCGTA GACAGTCTGG TCCTTGATAT GCTTCTTCCA	3900
AAGCAAATGA TTCTCCAAC TCGTCAAGTCA ATTTTGACAG AGAGACGTCT GGTGTTAGCT	3960
GGAGCAACTG GAATTGGAAA GAGCAAAC TCGAAGACCC TGGCTGCTTA TGTATCTATT	4020
CGAACAAATC AATCCGAAGA TAGTATTGTT AATATCAGCA TTCCTGAAAA CAATAAAGAA	4080
GAATTGCTTC AAGTGGAACG ACGCCTGGAA AAGATCTTGA GAAGCAAAGA ATCATGCATC	4140
GTAATTCTAG ATAATATCCC AAAGAATCGA ATTGCAATTTG TTGTATCCGT TTTTGCAAAT	4200
GTCCCACTTC AAAACAACGA AGGTCCATTT GTAGTATGCA CAGTCAACCG ATATCAAATC	4260
CCTGAGCTTC AAATTCACCA CAATTTCAAA ATGTCAGTAA TGTCGAATCG TCTCGAAGGA	4320
TTCATCCTAC GTTACCTCCG ACGACGGGCG GTAGAGGATG AGTATCGTCT AACTGTACAG	4380
ATGCCATCAG AGCTCTTCAA AATCATTGAC TTCTTCCCAA TAGCTCTTCA GGCCGTCAAT	4440
AATTTTATTG AGAAAACGAA TTCTGTTGAT GTGACAGTTG GTCCAAGAGC ATGCTTGAAC	4500
TGTCCTCTAA CTGTCGATGG ATCCCGTGAA TGGTTCATTC GATTGTGGAA TGAGAACTTC	4560
ATTCCATATT TGGAACGTGT TGCTAGAGAT GGCAAAAAAA ACCTTCGGTC GCTGCACTTC	4620
CTTCGAGGAT CCCACCGACA TCGTCTCTAA AAAATGGCCG TGGTTCGATG GTGAAAACCC	4680
GGAGAATGTG CTCAAACGTC TTCAACTCCA AGACCTCGTC CCGTCACCTG CCAACTCATC	4740
CCGACAACAC TTCAATCCCC TCGAGTCGTT GATCCAATTG CATGCTACCA AGCATCAGAC	4800
CATCGACAAC ATTTGAACAG AAGACTCTAA TCTTCTCTCG CCTCTCCCCC GCTTTCCTTA	4860
TCTTCGTACC GGTACCTGAT GATTCCCCAT TTTCCCCCTT TTCCCCCAA TTTCCAGAA	4920
CCTCCTGTTT CTTTGTTC TAGTCCTCCC GGGTGCCGAC GCCGAAGCGA TTTAAAAACC	4980
TTTTTCTTTC CGAAACATTT CCCATTGCTC ATTAATAGTC AAATTGAATA AACAGTGTAT	5040
GTACTTAAAA AAAAAAAAAA AAAAAAAAAA AAA	5073

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5072 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGTTTAATTA CCCAAGTTTG AGACATCAAT TCCATCGAAC GAAATGTTGG TGCTCCGAAT	60
AAAATGACGA CGTCAAATGT AGAATTGATA CCAATCTACA CGGATTGGGC CAATCGGCAC	120
CTTTCGAAGG GCAGCTTATC AAAGTCGATT AGGGATATTT CCAATGATTT TCGCGACTAT	180

CGACTGGTTT	CTCAGCTTAT	TAATGTGATC	GTTCCGATCA	ACGAATTCTC	GCCTGCATTTC	240
ACGAAACGTT	TGGCAAAAAT	CACATCGAAC	CTGGATGGCC	TCGAAACGTG	TCTCGACTAC	300
CTGAAAAATC	TGGGTCTCGA	CTGCTCGAAA	CTCACC AAAA	CCGATATCGA	CAGCGGAAAC	360
TTGGGTGCAG	TTCTCCAGCT	GCTCTTCCTG	CTCTCCACCT	ACAAGCAGAA	GCTTCGGCAA	420
CTGAAAAAAG	ATCAGAAGAA	ATTGGAGCAA	CTACCCACAT	CCATTATGCC	ACCCGCGGTT	480
TCTAAATTAC	CCTCGCCACG	TGTCGCCACG	TCAGCAACCG	CTTCAGCAAC	TAACCCAAAT	540
TCCAAC TTTC	CACAAATGTC	AACATCCAGG	CTTCAGACTC	CACAGTCAAG	AATATCGAAA	600
ATTGATTCAT	CAAAGATTGG	TATCAAGCCA	AAGACGTCTG	GACTTAAACC	ACCCTCATCA	660
TCAACCACTT	CATCAAATAA	TACAAATTCA	TTCCGTCCGT	CGAGCCGTTC	GAGTGGCAAT	720
AATAATGTTG	GCTCGACGAT	ATCCACATCT	GCGAAGAGCT	TAGAATCATC	ATCAACGTAC	780
AGCTCTATTT	CGAATCTAAA	CCGACCTACC	TCCCAACTCC	AAAAACCTTC	TAGACCACAA	840
ACCCAGCTAG	TTCGTGTTGC	TACAACTACA	AAAATCGGAA	GCTCAAAGCT	AGCCGCTCCG	900
AAAGCCGTGA	GCACCCCAAA	ACTTGCTTCT	GTGAAGACTA	TTGGAGCAAA	ACAAGAGCCC	960
GATAACAGCG	GTGGTGGTGG	TGGTGG AATG	CTGAAATTAA	AGTTATTTCAG	TAGCAAAAAC	1020
CCATCTTCCT	CATCGAATAG	CCCACAACCT	ACGAGAAAGG	CGGCGGCGGT	GCCTCAACAA	1080
CAAAC TTGT	CGAAAATCGC	TGCCCCAGTG	AAAAGTGGCC	TGAAGCCGCC	GACCAAGTAAG	1140
CTGGGAAGTG	CCACGTCTAT	GTCGAAGCTT	TGTACGCCAA	AAGTTTCCTA	CCGTAAAACG	1200
GACGCCCCAA	TCATATCTCA	ACAAGACTCG	AAACGATGCT	CAAAGAGCAG	TGAAGAAGAG	1260
TCCGGATACG	CTGGATTCAA	CAGCAGTCG	CCAACGTCAT	CATCGACGGA	AGGTTCCCTA	1320
AGCATGCATT	CCACATCTTC	CAAGAGTTCA	ACGTCAGACG	AAAAGTCTCC	GTCATCAGAC	1380
GATCTTACTC	TTAACGCCTC	CATCGTGACA	GCTATCAGAC	AGCCGATAGC	CGCAACACCG	1440
GTTTCTCCAA	ATATTATCAA	CAAGCCTGTT	GAGGAAAAAC	CAACACTGGC	AGTGAAAGGA	1500
GTGAAAAGCA	CAGCGAAAAA	AGATCCACCT	CCAGCTGTTC	CGCCACGTGA	CACCCAGCCA	1560
ACAATCGGAG	TTGTTAGTCC	AATTATGGCA	CATAAGAAGT	TGACAAATGA	CCCCGTGATA	1620
TCTGAAAAAC	CAGAACCTGA	AAAGCTCCAA	TCAATGAGCA	TCGACACGAC	GGACGTTCCA	1680
CCGCTTCCAC	CTCTAAAATC	AGTTGTTCCA	CTTAAAATGA	CTTCAATCCG	ACAACCACCA	1740
ACGTACGATG	TTCTTCTAAA	ACAAGGAAAA	ATCACATCGC	CTGTCAAGTC	GTTTGGATAT	1800
GAGCAGTCGT	CCGCGTCTGA	AGACTCCATT	GTGGCTCATG	CGTCGGCTCA	GGTGA CTCCG	1860
CCGACAAAAA	CTTCTGGTAA	TCATTGCTG	GAGAGAAGGA	TGGGAAAGAA	TAAGACATCA	1920
GAATCCAGCG	GCTACACCTC	TGACGCCGGT	GTTGCGATGT	GCGCCAAAAT	GAGGGAGAAAG	1980
CTGAAAAGAA	ACGATGACAT	GACTCGTCGA	GCACAGAACG	GCTATCCTGA	CAACTTCGAA	2040
GACAGTTCCT	CCTTGTCGTC	TGGAATATCC	GATAACAACG	AGCTCGACGA	CATATCCACG	2100

GACGATTGT	CCGGAGTAGA	CATGGCAACA	GTCGCCTCCA	AACATAGCGA	CTATTCCCAC	2160
TTTGTTCGCC	ATCCCACGTC	TTCTTCCTCA	AAGCCCCGAG	TCCCCAGTCG	GTCCTCCACA	2220
TCAGTCGATT	CTCGATCTCG	AGCAGAACAG	GAGAATGTGT	ACAAACTTCT	GTCCCAGTGC	2280
CGAACGAGCC	AACGTGGCGC	CGCTGCCACC	TCAACCTTCG	GACAACATTC	GCTAAGATCC	2340
CCGGGATACT	CATCCTATTC	TCCACACTTA	TCAGTGTGAG	CTGATAAGGA	CACAATGTCT	2400
ATGCACTCAC	AGACTAGTCG	ACGACCTTCT	TCACAAAAAC	CAAGCTATTC	AGGCCAATTT	2460
CATTCACTTG	ATCGTAAATG	CCACCTTCAA	GAGTTCACAT	CCACCGAGCA	CAGAATGGCG	2520
GCTCTCTTGA	GCCCCGAGACG	GGTGCCGAAC	TCGATGTGCA	AATATGATTC	TTCAGGATCC	2580
TACTCGGCGC	GTTCCCGAGG	TGGAAGCTCT	ACTGGTATCT	ATGGAGAGAC	GTTCCAAGTG	2640
CACAGACTAT	CCGATGAAAA	ATCCCCCGCA	CATTCTGCCA	AAAGTGAGAT	GGGATCCCAA	2700
CTATCACTGG	CTAGCACGAC	AGCATATGGA	TCTCTCAATG	AGAAGTACGA	ACATGCTATT	2760
CGGGACATGG	CACGTGACTT	GGAGTGTTAC	AAGAACACTG	TCGACTCACT	AACCAAGAAA	2820
CAGGAGAACT	ATGGAGCATT	GTTTGATCTT	TTTGAGCAAA	AGCTTAGAAA	ACTCACTCAA	2880
CACATTGATC	GATCCAACCT	GAAGCCTGAA	GAGGCAATAC	GATTGAGGCA	GGACATTGCT	2940
CATTTGAGGG	ATATTAGCAA	TCATCTTGCA	TCCAACCTCAG	CTCATGCTAA	CGAAGGCGCT	3000
GGTGAGCTTC	TTCGTCAACC	ATCTCTGGAA	TCAGTTGCAT	CCCATCGATC	ATCGATGTCA	3060
TCGTGTCGA	AAAGCAGCAA	GCAGGAGAAG	ATCAGCTTGA	GCTCGTTTGG	CAAGAACAAG	3120
AAGAGCTGGA	TCCGCTCCTC	ACTCTCCAAG	TTCACCAAGA	AGAAGAACAA	GAAGTACGAC	3180
GAAGCACATA	TGCCATCAAT	TTCCGGATCT	CAAGGAACCT	TTGACAACAT	TGATGTGATT	3240
GAGTTGAAGC	AAGAGCTCAA	AGAACGCGAT	AGTGCACTTT	ACGAAGTCCG	CCTTGACAAT	3300
CTGGATCGTG	CCCGCGAAGT	TGATGTTCTG	AGGGAGACAG	TGAACAAGTT	GAAAACCGAG	3360
AACAAGCAAT	TAAAGAAAGA	AGTGGACAAA	CTCACCAACG	GTCCAGCCAC	TCGTGCTTCT	3420
TCCCGCGCCT	CAATTCCAGT	TATCTACGAC	GATGAGCATG	TCTATGATGC	AGCGTGTAGC	3480
AGTACATCAG	CTAGTCAATC	TTCGAAACGA	TCCTCTGGCT	GCAACTCAAT	CAAGGTTACT	3540
GTAAACGTGG	ACATCGCTGG	AGAAATCAGT	TCGATCGTTA	ACCCGGACAA	AGAGATAATC	3600
GTAGGATATC	TTGCCATGTC	AACCAGTCAG	TCATGCTGGA	AAGACATTGA	TGTTTCTATT	3660
CTAGGACTAT	TTGAAGTCTA	CCTATCCAGA	ATTGATGTGG	AGCATCAACT	TGGAATCGAT	3720
GCTCGTGATT	CTATCCTTGG	CTATCAAATT	GGTGAACCTC	GACGCGTCAT	TGGAGACTCC	3780
ACAACCATGA	TAACCAGCCA	TCCAACCTGAC	ATTCTTACTT	CCTCAACTAC	AATCCGAATG	3840
TTCATGCACG	GTGCCGCACA	GAGTCGCGTA	GACAGTCTGG	TCCTTGATAT	GCTTCTTCCA	3900
AAGCAAATGA	TTCTCCAACCT	CGTCAAGTCA	ATTTTGACAG	AGAGACGTCT	GGTGTAGCT	3960
GGAGCAACTG	GAATTGGAAA	GAGCAAACTG	GCGAAGACCC	TGGCTGCTTA	TGTATCTATT	4020

CGAACAAATC AATCCGAAGA TAGTATTGTT AATATCAGCA TTCCTGAAAA CAATAAAGAA 4080  
GAATTGCTTC AAGTGGAAACG ACGCCTGGAA AAGATCTTGA GAAGCAAAGA ATCATGCATC 4140  
GTAATTCTAG ATAATATCCC AAAGAATCGA ATTGCATTTG TTGTATCCGT TTTTGCAAAT 4200  
GTCCCACTTC AAAACAACGA AGGTCCATTT GTAGTATGCA CAGTCAACCG ATATCAAATC 4260  
CCTGAGCTTC AAATTCACCA CAATTTCAAA ATGTCAGTAA TGTCGAATCG TCTCGAAGGA 4320  
TTCATCCTAC GTTACCTCCG ACGACGGGCG GTAGAGGATG AGTATCGTCT AACTGTACAG 4380  
ATGCCATCAG AGCTCTTCAA AATCATTGAC TTCTTCCCAA TAGCTCTTCA GGCCGTCAAT 4440  
AATTTTATTG AGAAAACGAA TTCTGTTGAT GTGACAGTTG GTCCAAGAGC ATGCTTGAAC 4500  
TGTCCTCTAA CTGTCGATGG ATCCCGTGAA TGGTTCATTC GATTGTGGAA TGAGAACTTC 4560  
ATTCCATATT TGGAACGTGT TGCTAGAGAT GGCAAAAAAA CCTTCGGTCG CTGCACTTCC 4620  
TTCGAGGATC CCACCGACAT CGTCTCTAAA AAATGGCCGT GGTTCGATGG TGAAAACCCG 4680  
GAGAATGTGC TCAAACGTCT TCAACTCCAA GACCTCGTCC CGTCACCTGC CAACTCATCC 4740  
CGACAACACT TCAATCCCCT CGAGTCGTTG ATCCAATTGC ATGCTACCAA GCATCAGACC 4800  
ATCGACAACA TTTGAACAGA AGACTCTAAT CTTCTCTCGC CTCTCCCCCG CTTTCCTTAT 4860  
CTTCGTACCG GTACCTGATG ATTCCCCATT TTCCCCCTTT TCCCCCAAT TTCCCAGAAC 4920  
CTCCTGTTCC CTTTGTTCTT AGTCCTCCCG GGTGCCGACG CCGAAGCGAT TTA AAAACCT 4980  
TTTTCTTTCC GAAACATTTT CCATTGCTCA TTAATAGTCA AATTGAATAA ACAGTGTATG 5040  
TACTTAAAAA AAAAAAAAAA AAAAAAAAAA AA 5072

## (2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1528 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp Ala  
1 5 10 15  
Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg Asp Ile  
20 25 30  
Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln Leu Ile Asn Val  
35 40 45  
Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr Lys Arg Leu Ala  
50 55 60



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Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr Cys Leu Asp Tyr Leu  
 65 70 75 80  
 Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu Thr Lys Thr Asp Ile Asp  
 85 90 95  
 Ser Gly Asn Leu Gly Ala Val Leu Gln Leu Leu Phe Leu Leu Ser Thr  
 100 105 110  
 Tyr Lys Gln Lys Leu Arg Gln Leu Lys Lys Asp Gln Lys Lys Leu Glu  
 115 120 125  
 Gln Leu Pro Thr Ser Ile Met Pro Pro Ala Val Ser Lys Leu Pro Ser  
 130 135 140  
 Pro Arg Val Ala Thr Ser Ala Thr Ala Ser Ala Thr Asn Pro Asn Ser  
 145 150 155 160  
 Asn Phe Pro Gln Met Ser Thr Ser Arg Leu Gln Thr Pro Gln Ser Arg  
 165 170 175  
 Ile Ser Lys Ile Asp Ser Ser Lys Ile Gly Ile Lys Pro Lys Thr Ser  
 180 185 190  
 Gly Leu Lys Pro Pro Ser Ser Ser Thr Thr Ser Ser Asn Asn Thr Asn  
 195 200 205  
 Ser Phe Arg Pro Ser Ser Arg Ser Ser Gly Asn Asn Asn Val Gly Ser  
 210 215 220  
 Thr Ile Ser Thr Ser Ala Lys Ser Leu Glu Ser Ser Ser Thr Tyr Ser  
 225 230 235 240  
 Ser Ile Ser Asn Leu Asn Arg Pro Thr Ser Gln Leu Gln Lys Pro Ser  
 245 250 255  
 Arg Pro Gln Thr Gln Leu Val Arg Val Ala Thr Thr Thr Lys Ile Gly  
 260 265 270  
 Ser Ser Lys Leu Ala Ala Pro Lys Ala Val Ser Thr Pro Lys Leu Ala  
 275 280 285  
 Ser Val Lys Thr Ile Gly Ala Lys Gln Glu Pro Asp Asn Ser Gly Gly  
 290 295 300  
 Gly Gly Gly Gly Met Leu Lys Leu Lys Leu Phe Ser Ser Lys Asn Pro  
 305 310 315 320  
 Ser Ser Ser Ser Asn Ser Pro Gln Pro Thr Arg Lys Ala Ala Ala Val  
 325 330 335  
 Pro Gln Gln Gln Thr Leu Ser Lys Ile Ala Ala Pro Val Lys Ser Gly  
 340 345 350  
 Leu Lys Pro Pro Thr Ser Lys Leu Gly Ser Ala Thr Ser Met Ser Lys  
 355 360 365  
 Leu Cys Thr Pro Lys Val Ser Tyr Arg Lys Thr Asp Ala Pro Ile Ile  
 370 375 380  
 Ser Gln Gln Asp Ser Lys Arg Cys Ser Lys Ser Ser Glu Glu Glu Ser  
 385 390 395 400

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Gly	Tyr	Ala	Gly	Phe	Asn	Ser	Thr	Ser	Pro	Thr	Ser	Ser	Ser	Thr	Glu	405	410	415	
Gly	Ser	Leu	Ser	Met	His	Ser	Thr	Ser	Ser	Lys	Ser	Ser	Thr	Ser	Asp	420	425	430	
Glu	Lys	Ser	Pro	Ser	Ser	Asp	Asp	Leu	Thr	Leu	Asn	Ala	Ser	Ile	Val	435	440	445	
Thr	Ala	Ile	Arg	Gln	Pro	Ile	Ala	Ala	Thr	Pro	Val	Ser	Pro	Asn	Ile	450	455	460	
Ile	Asn	Lys	Pro	Val	Glu	Glu	Lys	Pro	Thr	Leu	Ala	Val	Lys	Gly	Val	465	470	475	480
Lys	Ser	Thr	Ala	Lys	Lys	Asp	Pro	Pro	Pro	Ala	Val	Pro	Pro	Arg	Asp	485	490	495	
Thr	Gln	Pro	Thr	Ile	Gly	Val	Val	Ser	Pro	Ile	Met	Ala	His	Lys	Lys	500	505	510	
Leu	Thr	Asn	Asp	Pro	Val	Ile	Ser	Glu	Lys	Pro	Glu	Pro	Glu	Lys	Leu	515	520	525	
Gln	Ser	Met	Ser	Ile	Asp	Thr	Thr	Asp	Val	Pro	Pro	Leu	Pro	Pro	Leu	530	535	540	
Lys	Ser	Val	Val	Pro	Leu	Lys	Met	Thr	Ser	Ile	Arg	Gln	Pro	Pro	Thr	545	550	555	560
Tyr	Asp	Val	Leu	Leu	Lys	Gln	Gly	Lys	Ile	Thr	Ser	Pro	Val	Lys	Ser	565	570	575	
Phe	Gly	Tyr	Glu	Gln	Ser	Ser	Ala	Ser	Glu	Asp	Ser	Ile	Val	Ala	His	580	585	590	
Ala	Ser	Ala	Gln	Val	Thr	Pro	Pro	Thr	Lys	Thr	Ser	Gly	Asn	His	Ser	595	600	605	
Leu	Glu	Arg	Arg	Met	Gly	Lys	Asn	Lys	Thr	Ser	Glu	Ser	Ser	Gly	Tyr	610	615	620	
Thr	Ser	Asp	Ala	Gly	Val	Ala	Met	Cys	Ala	Lys	Met	Arg	Glu	Lys	Leu	625	630	635	640
Lys	Glu	Tyr	Asp	Asp	Met	Thr	Arg	Arg	Ala	Gln	Asn	Gly	Tyr	Pro	Asp	645	650	655	
Asn	Phe	Glu	Asp	Ser	Ser	Ser	Leu	Ser	Ser	Gly	Ile	Ser	Asp	Asn	Asn	660	665	670	
Glu	Leu	Asp	Asp	Ile	Ser	Thr	Asp	Asp	Leu	Ser	Gly	Val	Asp	Met	Ala	675	680	685	
Thr	Val	Ala	Ser	Lys	His	Ser	Asp	Tyr	Ser	His	Phe	Val	Arg	His	Pro	690	695	700	
Thr	Ser	Ser	Ser	Ser	Lys	Pro	Arg	Val	Pro	Ser	Arg	Ser	Ser	Thr	Ser	705	710	715	720
Val	Asp	Ser	Arg	Ser	Arg	Ala	Glu	Gln	Glu	Asn	Val	Tyr	Lys	Leu	Leu	725	730	735	

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Ser Gln Cys Arg Thr Ser Gln Arg Gly Ala Ala Ala Thr Ser Thr Phe  
 740 745 750  
 Gly Gln His Ser Leu Arg Ser Pro Gly Tyr Ser Ser Tyr Ser Pro His  
 755 760 765  
 Leu Ser Val Ser Ala Asp Lys Asp Thr Met Ser Met His Ser Gln Thr  
 770 775 780  
 Ser Arg Arg Pro Ser Ser Gln Lys Pro Ser Tyr Ser Gly Gln Phe His  
 785 790 795 800  
 Ser Leu Asp Arg Lys Cys His Leu Gln Glu Phe Thr Ser Thr Glu His  
 805 810 815  
 Arg Met Ala Ala Leu Leu Ser Pro Arg Arg Val Pro Asn Ser Met Ser  
 820 825 830  
 Lys Tyr Asp Ser Ser Gly Ser Tyr Ser Ala Arg Ser Arg Gly Gly Ser  
 835 840 845  
 Ser Thr Gly Ile Tyr Gly Glu Thr Phe Gln Leu His Arg Leu Ser Asp  
 850 855 860  
 Glu Lys Ser Pro Ala His Ser Ala Lys Ser Glu Met Gly Ser Gln Leu  
 865 870 875 880  
 Ser Leu Ala Ser Thr Thr Ala Tyr Gly Ser Leu Asn Glu Lys Tyr Glu  
 885 890 895  
 His Ala Ile Arg Asp Met Ala Arg Asp Leu Glu Cys Tyr Lys Asn Thr  
 900 905 910  
 Val Asp Ser Leu Thr Lys Lys Gln Glu Asn Tyr Gly Ala Leu Phe Asp  
 915 920 925  
 Leu Phe Glu Gln Lys Leu Arg Lys Leu Thr Gln His Ile Asp Arg Ser  
 930 935 940  
 Asn Leu Lys Pro Glu Glu Ala Ile Arg Phe Arg Gln Asp Ile Ala His  
 945 950 955 960  
 Leu Arg Asp Ile Ser Asn His Leu Ala Ser Asn Ser Ala His Ala Asn  
 965 970 975  
 Glu Gly Ala Gly Glu Leu Leu Arg Gln Pro Ser Leu Glu Ser Val Ala  
 980 985 990  
 Ser His Arg Ser Ser Met Ser Ser Ser Ser Lys Ser Ser Lys Gln Glu  
 995 1000 1005  
 Lys Ile Ser Leu Ser Ser Phe Gly Lys Asn Lys Lys Ser Trp Ile Arg  
 1010 1015 1020  
 Ser Ser Leu Ser Lys Phe Thr Lys Lys Lys Asn Lys Asn Tyr Asp Glu  
 1025 1030 1035 1040  
 Ala His Met Pro Ser Ile Ser Gly Ser Gln Gly Thr Leu Asp Asn Ile  
 1045 1050 1055  
 Asp Val Ile Glu Leu Lys Gln Glu Leu Lys Glu Arg Asp Ser Ala Leu  
 1060 1065 1070

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Tyr Glu Val Arg Leu Asp Asn Leu Asp Arg Ala Arg Glu Val Asp Val  
 1075 1080 1085  
 Leu Arg Glu Thr Val Asn Lys Leu Lys Thr Glu Asn Lys Gln Leu Lys  
 1090 1095 1100  
 Lys Glu Val Asp Lys Leu Thr Asn Gly Pro Ala Thr Arg Ala Ser Ser  
 1105 1110 1115 1120  
 Arg Ala Ser Ile Pro Val Ile Tyr Asp Asp Glu His Val Tyr Asp Ala  
 1125 1130 1135  
 Ala Cys Ser Ser Thr Ser Ala Ser Gln Ser Ser Lys Arg Ser Ser Gly  
 1140 1145 1150  
 Cys Asn Ser Ile Lys Val Thr Val Asn Val Asp Ile Ala Gly Glu Ile  
 1155 1160 1165  
 Ser Ser Ile Val Asn Pro Asp Lys Glu Ile Ile Val Gly Tyr Leu Ala  
 1170 1175 1180  
 Met Ser Thr Ser Gln Ser Cys Trp Lys Asp Ile Asp Val Ser Ile Leu  
 1185 1190 1195 1200  
 Gly Leu Phe Glu Val Tyr Leu Ser Arg Ile Asp Val Glu His Gln Leu  
 1205 1210 1215  
 Gly Ile Asp Ala Arg Asp Ser Ile Leu Gly Tyr Gln Ile Gly Glu Leu  
 1220 1225 1230  
 Arg Arg Val Ile Gly Asp Ser Thr Thr Met Ile Thr Ser His Pro Thr  
 1235 1240 1245  
 Asp Ile Leu Thr Ser Ser Thr Thr Ile Arg Met Phe Met His Gly Ala  
 1250 1255 1260  
 Ala Gln Ser Arg Val Asp Ser Leu Val Leu Asp Met Leu Leu Pro Lys  
 1265 1270 1275 1280  
 Gln Met Ile Leu Gln Leu Val Lys Ser Ile Leu Thr Glu Arg Arg Leu  
 1285 1290 1295  
 Val Leu Ala Gly Ala Thr Gly Ile Gly Lys Ser Lys Leu Ala Lys Thr  
 1300 1305 1310  
 Leu Ala Ala Tyr Val Ser Ile Arg Thr Asn Gln Ser Glu Asp Ser Ile  
 1315 1320 1325  
 Val Asn Ile Ser Ile Pro Glu Asn Asn Lys Glu Glu Leu Leu Gln Val  
 1330 1335 1340  
 Glu Arg Arg Leu Glu Lys Ile Leu Arg Ser Lys Glu Ser Cys Ile Val  
 1345 1350 1355 1360  
 Ile Leu Asp Asn Ile Pro Lys Asn Arg Ile Ala Phe Val Val Ser Val  
 1365 1370 1375  
 Phe Ala Asn Val Pro Leu Gln Asn Asn Glu Gly Pro Phe Val Val Cys  
 1380 1385 1390  
 Thr Val Asn Arg Tyr Gln Ile Pro Glu Leu Gln Ile His His Asn Phe  
 1395 1400 1405

111

Lys Met Ser Val Met Ser Asn Arg Leu Glu Gly Phe Ile Leu Arg Tyr  
 1410 1415 1420  
 Leu Arg Arg Arg Ala Val Glu Asp Glu Tyr Arg Leu Thr Val Gln Met  
 1425 1430 1435 1440  
 Pro Ser Glu Leu Phe Lys Ile Ile Asp Phe Phe Pro Ile Ala Leu Gln  
 1445 1450 1455  
 Ala Val Asn Asn Phe Ile Glu Lys Thr Asn Ser Val Asp Val Thr Val  
 1460 1465 1470  
 Gly Pro Arg Ala Cys Leu Asn Cys Pro Leu Thr Val Asp Gly Ser Arg  
 1475 1480 1485  
 Glu Trp Phe Ile Arg Leu Trp Asn Glu Asn Phe Ile Pro Tyr Leu Glu  
 1490 1495 1500  
 Arg Val Ala Arg Asp Gly Lys Lys Asn Leu Arg Ser Leu His Phe Leu  
 1505 1510 1515 1520  
 Arg Gly Ser His Arg His Arg Leu  
 1525

## (2) INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1583 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp Ala  
 1 5 10 15  
 Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg Asp Ile  
 20 25 30  
 Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln Leu Ile Asn Val  
 35 40 45  
 Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr Lys Arg Leu Ala  
 50 55 60  
 Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr Cys Leu Asp Tyr Leu  
 65 70 75 80  
 Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu Thr Lys Thr Asp Ile Asp  
 85 90 95  
 Ser Gly Asn Leu Gly Ala Val Leu Gln Leu Leu Phe Leu Leu Ser Thr  
 100 105 110  
 Tyr Lys Gln Lys Leu Arg Gln Leu Lys Lys Asp Gln Lys Lys Leu Glu  
 115 120 125

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Gln	Leu	Pro	Thr	Ser	Ile	Met	Pro	Pro	Ala	Val	Ser	Lys	Leu	Pro	Ser	130	135	140
Pro	Arg	Val	Ala	Thr	Ser	Ala	Thr	Ala	Ser	Ala	Thr	Asn	Pro	Asn	Ser	145	150	155
Asn	Phe	Pro	Gln	Met	Ser	Thr	Ser	Arg	Leu	Gln	Thr	Pro	Gln	Ser	Arg	165	170	175
Ile	Ser	Lys	Ile	Asp	Ser	Ser	Lys	Ile	Gly	Ile	Lys	Pro	Lys	Thr	Ser	180	185	190
Gly	Leu	Lys	Pro	Pro	Ser	Ser	Ser	Thr	Thr	Ser	Ser	Asn	Asn	Thr	Asn	195	200	205
Ser	Phe	Arg	Pro	Ser	Ser	Arg	Ser	Ser	Gly	Asn	Asn	Asn	Val	Gly	Ser	210	215	220
Thr	Ile	Ser	Thr	Ser	Ala	Lys	Ser	Leu	Glu	Ser	Ser	Ser	Thr	Tyr	Ser	225	230	235
Ser	Ile	Ser	Asn	Leu	Asn	Arg	Pro	Thr	Ser	Gln	Leu	Gln	Lys	Pro	Ser	245	250	255
Arg	Pro	Gln	Thr	Gln	Leu	Val	Arg	Val	Ala	Thr	Thr	Thr	Lys	Ile	Gly	260	265	270
Ser	Ser	Lys	Leu	Ala	Ala	Pro	Lys	Ala	Val	Ser	Thr	Pro	Lys	Leu	Ala	275	280	285
Ser	Val	Lys	Thr	Ile	Gly	Ala	Lys	Gln	Glu	Pro	Asp	Asn	Ser	Gly	Gly	290	295	300
Gly	Gly	Gly	Gly	Met	Leu	Lys	Leu	Lys	Leu	Phe	Ser	Ser	Lys	Asn	Pro	305	310	315
Ser	Ser	Ser	Ser	Asn	Ser	Pro	Gln	Pro	Thr	Arg	Lys	Ala	Ala	Ala	Val	325	330	335
Pro	Gln	Gln	Gln	Thr	Leu	Ser	Lys	Ile	Ala	Ala	Pro	Val	Lys	Ser	Gly	340	345	350
Leu	Lys	Pro	Pro	Thr	Ser	Lys	Leu	Gly	Ser	Ala	Thr	Ser	Met	Ser	Lys	355	360	365
Leu	Cys	Thr	Pro	Lys	Val	Ser	Tyr	Arg	Lys	Thr	Asp	Ala	Pro	Ile	Ile	370	375	380
Ser	Gln	Gln	Asp	Ser	Lys	Arg	Cys	Ser	Lys	Ser	Ser	Glu	Glu	Glu	Ser	385	390	395
Gly	Tyr	Ala	Gly	Phe	Asn	Ser	Thr	Ser	Pro	Thr	Ser	Ser	Ser	Thr	Glu	405	410	415
Gly	Ser	Leu	Ser	Met	His	Ser	Thr	Ser	Ser	Lys	Ser	Ser	Thr	Ser	Asp	420	425	430
Glu	Lys	Ser	Pro	Ser	Ser	Asp	Asp	Leu	Thr	Leu	Asn	Ala	Ser	Ile	Val	435	440	445
Thr	Ala	Ile	Arg	Gln	Pro	Ile	Ala	Ala	Thr	Pro	Val	Ser	Pro	Asn	Ile	450	455	460

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Ile Asn Lys Pro Val Glu Glu Lys Pro Thr Leu Ala Val Lys Gly Val  
 465 470 475 480  
 Lys Ser Thr Ala Lys Lys Asp Pro Pro Pro Ala Val Pro Pro Arg Asp  
 485 490 495  
 Thr Gln Pro Thr Ile Gly Val Val Ser Pro Ile Met Ala His Lys Lys  
 500 505 510  
 Leu Thr Asn Asp Pro Val Ile Ser Glu Lys Pro Glu Pro Glu Lys Leu  
 515 520 525  
 Gln Ser Met Ser Ile Asp Thr Thr Asp Val Pro Pro Leu Pro Pro Leu  
 530 535 540  
 Lys Ser Val Val Pro Leu Lys Met Thr Ser Ile Arg Gln Pro Pro Thr  
 545 550 555 560  
 Tyr Asp Val Leu Leu Lys Gln Gly Lys Ile Thr Ser Pro Val Lys Ser  
 565 570 575  
 Phe Gly Tyr Glu Gln Ser Ser Ala Ser Glu Asp Ser Ile Val Ala His  
 580 585 590  
 Ala Ser Ala Gln Val Thr Pro Pro Thr Lys Thr Ser Gly Asn His Ser  
 595 600 605  
 Leu Glu Arg Arg Met Gly Lys Asn Lys Thr Ser Glu Ser Ser Gly Tyr  
 610 615 620  
 Thr Ser Asp Ala Gly Val Ala Met Cys Ala Lys Met Arg Glu Lys Leu  
 625 630 635 640  
 Lys Glu Tyr Asp Asp Met Thr Arg Arg Ala Gln Asn Gly Tyr Pro Asp  
 645 650 655  
 Asn Phe Glu Asp Ser Ser Ser Leu Ser Ser Gly Ile Ser Asp Asn Asn  
 660 665 670  
 Glu Leu Asp Asp Ile Ser Thr Asp Asp Leu Ser Gly Val Asp Met Ala  
 675 680 685  
 Thr Val Ala Ser Lys His Ser Asp Tyr Ser His Phe Val Arg His Pro  
 690 695 700  
 Thr Ser Ser Ser Ser Lys Pro Arg Val Pro Ser Arg Ser Ser Thr Ser  
 705 710 715 720  
 Val Asp Ser Arg Ser Arg Ala Glu Gln Glu Asn Val Tyr Lys Leu Leu  
 725 730 735  
 Ser Gln Cys Arg Thr Ser Gln Arg Gly Ala Ala Ala Thr Ser Thr Phe  
 740 745 750  
 Gly Gln His Ser Leu Arg Ser Pro Gly Tyr Ser Ser Tyr Ser Pro His  
 755 760 765  
 Leu Ser Val Ser Ala Asp Lys Asp Thr Met Ser Met His Ser Gln Thr  
 770 775 780  
 Ser Arg Arg Pro Ser Ser Gln Lys Pro Ser Tyr Ser Gly Gln Phe His  
 785 790 795 800

114

Ser Leu Asp Arg Lys Cys His Leu Gln Glu Phe Thr Ser Thr Glu His  
 805 810 815  
 Arg Met Ala Ala Leu Leu Ser Pro Arg Arg Val Pro Asn Ser Met Ser  
 820 825 830  
 Lys Tyr Asp Ser Ser Gly Ser Tyr Ser Ala Arg Ser Arg Gly Gly Ser  
 835 840 845  
 Ser Thr Gly Ile Tyr Gly Glu Thr Phe Gln Leu His Arg Leu Ser Asp  
 850 855 860  
 Glu Lys Ser Pro Ala His Ser Ala Lys Ser Glu Met Gly Ser Gln Leu  
 865 870 875 880  
 Ser Leu Ala Ser Thr Thr Ala Tyr Gly Ser Leu Asn Glu Lys Tyr Glu  
 885 890 895  
 His Ala Ile Arg Asp Met Ala Arg Asp Leu Glu Cys Tyr Lys Asn Thr  
 900 905 910  
 Val Asp Ser Leu Thr Lys Lys Gln Glu Asn Tyr Gly Ala Leu Phe Asp  
 915 920 925  
 Leu Phe Glu Gln Lys Leu Arg Lys Leu Thr Gln His Ile Asp Arg Ser  
 930 935 940  
 Asn Leu Lys Pro Glu Glu Ala Ile Arg Phe Arg Gln Asp Ile Ala His  
 945 950 955 960  
 Leu Arg Asp Ile Ser Asn His Leu Ala Ser Asn Ser Ala His Ala Asn  
 965 970 975  
 Glu Gly Ala Gly Glu Leu Leu Arg Gln Pro Ser Leu Glu Ser Val Ala  
 980 985 990  
 Ser His Arg Ser Ser Met Ser Ser Ser Ser Lys Ser Ser Lys Gln Glu  
 995 1000 1005  
 Lys Ile Ser Leu Ser Ser Phe Gly Lys Asn Lys Lys Ser Trp Ile Arg  
 1010 1015 1020  
 Ser Ser Leu Ser Lys Phe Thr Lys Lys Lys Asn Lys Asn Tyr Asp Glu  
 1025 1030 1035 1040  
 Ala His Met Pro Ser Ile Ser Gly Ser Gln Gly Thr Leu Asp Asn Ile  
 1045 1050 1055  
 Asp Val Ile Glu Leu Lys Gln Glu Leu Lys Glu Arg Asp Ser Ala Leu  
 1060 1065 1070  
 Tyr Glu Val Arg Leu Asp Asn Leu Asp Arg Ala Arg Glu Val Asp Val  
 1075 1080 1085  
 Leu Arg Glu Thr Val Asn Lys Leu Lys Thr Glu Asn Lys Gln Leu Lys  
 1090 1095 1100  
 Lys Glu Val Asp Lys Leu Thr Asn Gly Pro Ala Thr Arg Ala Ser Ser  
 1105 1110 1115 1120  
 Arg Ala Ser Ile Pro Val Ile Tyr Asp Asp Glu His Val Tyr Asp Ala  
 1125 1130 1135



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Ala Cys Ser Ser Thr Ser Ala Ser Gln Ser Ser Lys Arg Ser Ser Gly  
 1140 1145 1150

Cys Asn Ser Ile Lys Val Thr Val Asn Val Asp Ile Ala Gly Glu Ile  
 1155 1160 1165

Ser Ser Ile Val Asn Pro Asp Lys Glu Ile Ile Val Gly Tyr Leu Ala  
 1170 1175 1180

Met Ser Thr Ser Gln Ser Cys Trp Lys Asp Ile Asp Val Ser Ile Leu  
 1185 1190 1195 1200

Gly Leu Phe Glu Val Tyr Leu Ser Arg Ile Asp Val Glu His Gln Leu  
 1205 1210 1215

Gly Ile Asp Ala Arg Asp Ser Ile Leu Gly Tyr Gln Ile Gly Glu Leu  
 1220 1225 1230

Arg Arg Val Ile Gly Asp Ser Thr Thr Met Ile Thr Ser His Pro Thr  
 1235 1240 1245

Asp Ile Leu Thr Ser Ser Thr Thr Ile Arg Met Phe Met His Gly Ala  
 1250 1255 1260

Ala Gln Ser Arg Val Asp Ser Leu Val Leu Asp Met Leu Leu Pro Lys  
 1265 1270 1275 1280

Gln Met Ile Leu Gln Leu Val Lys Ser Ile Leu Thr Glu Arg Arg Leu  
 1285 1290 1295

Val Leu Ala Gly Ala Thr Gly Ile Gly Lys Ser Lys Leu Ala Lys Thr  
 1300 1305 1310

Leu Ala Ala Tyr Val Ser Ile Arg Thr Asn Gln Ser Glu Asp Ser Ile  
 1315 1320 1325

Val Asn Ile Ser Ile Pro Glu Asn Asn Lys Glu Glu Leu Leu Gln Val  
 1330 1335 1340

Glu Arg Arg Leu Glu Lys Ile Leu Arg Ser Lys Glu Ser Cys Ile Val  
 1345 1350 1355 1360

Ile Leu Asp Asn Ile Pro Lys Asn Arg Ile Ala Phe Val Val Ser Val  
 1365 1370 1375

Phe Ala Asn Val Pro Leu Gln Asn Asn Glu Gly Pro Phe Val Val Cys  
 1380 1385 1390

Thr Val Asn Arg Tyr Gln Ile Pro Glu Leu Gln Ile His His Asn Phe  
 1395 1400 1405

Lys Met Ser Val Met Ser Asn Arg Leu Glu Gly Phe Ile Leu Arg Tyr  
 1410 1415 1420

Leu Arg Arg Arg Ala Val Glu Asp Glu Tyr Arg Leu Thr Val Gln Met  
 1425 1430 1435 1440

Pro Ser Glu Leu Phe Lys Ile Ile Asp Phe Phe Pro Ile Ala Leu Gln  
 1445 1450 1455

Ala Val Asn Asn Phe Ile Glu Lys Thr Asn Ser Val Asp Val Thr Val  
 1460 1465 1470

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Gly Pro Arg Ala Cys Leu Asn Cys Pro Leu Thr Val Asp Gly Ser Arg  
 1475 1480 1485

Glu Trp Phe Ile Arg Leu Trp Asn Glu Asn Phe Ile Pro Tyr Leu Glu  
 1490 1495 1500

Arg Val Ala Arg Asp Gly Lys Lys Thr Phe Gly Arg Cys Thr Ser Phe  
 1505 1510 1515 1520

Glu Asp Pro Thr Asp Ile Val Ser Lys Lys Trp Pro Trp Phe Asp Gly  
 1525 1530 1535

Glu Asn Pro Glu Asn Val Leu Lys Arg Leu Gln Leu Gln Asp Leu Val  
 1540 1545 1550

Pro Ser Pro Ala Asn Ser Ser Arg Gln His Phe Asn Pro Leu Glu Ser  
 1555 1560 1565

Leu Ile Gln Leu His Ala Thr Lys His Gln Thr Ile Asp Asn Ile  
 1570 1575 1580

## (2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 47 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

ATAAGAATGC GGCCGCCGCC ATGACGACGT CAAATGTAGA ATTGATA

47

## (2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 41 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGAATTCCAA CCATATGACG ACGTCAAATG TAGAATTGAT A

41

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## (2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CGCGGATCCT CAAACCGCGG GTGGCATAAT GGATG

35

## (2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 13 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Lys	Lys	Asp	Pro	Pro	Pro	Ala	Val	Pro	Pro	Arg	Asp	Thr
1				5						10		

## (2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Thr	Thr	Asp	Val	Pro	Pro	Leu	Pro	Pro	Leu	Lys	Ser
1				5					10		

## (2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Glu Val Pro Val Pro Pro Pro Val Pro Pro Arg Arg  
1 5 10

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

His Leu Asp Ser Pro Pro Ala Ile Pro Pro Arg  
1 5 10

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

His Ser Ile Ala Gly Pro Pro Val Pro Pro Arg  
1 5 10

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Tyr Arg Ala Val Pro Pro Pro Leu Pro Pro Arg Arg Lys  
1 5 10

## (2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Gly Glu Leu Ser Pro Pro Pro Ile Pro Pro Arg Leu Asn  
1 5 10

## (2) INFORMATION FOR SEQ ID NO: 15:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Ala Pro Ala Val Pro Pro Ala Arg Pro Gly Ser  
1 5 10

## (2) INFORMATION FOR SEQ ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Pro Ala Val Pro Pro Ala Arg Pro  
1 5

## (2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Pro	Pro	Arg	Pro	Leu	Pro	Val	Ala	Pro	Gly	Ser
1				5					10	

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 10 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Pro	Ala	Pro	Ala	Pro	Pro	Lys	Pro	Pro	Lys
1				5					10

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 13 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Pro	Pro	Asp	Asn	Gly	Pro	Pro	Pro	Leu	Pro	Thr	Ser	Ser
1				5					10			

(2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 13 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Pro	Pro	Gln	Met	Pro	Leu	Pro	Glu	Ile	Pro	Gln	Gln	Trp
1				5					10			

(2) INFORMATION FOR SEQ ID NO: 21:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 13 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Ala	Pro	Thr	Met	Pro	Pro	Pro	Leu	Pro	Pro	Val	Pro	Pro
1				5						10		

(2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 12 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Phe	Pro	Ala	Tyr	Pro	Pro	Pro	Pro	Val	Pro	Val	Pro
1				5						10	

(2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 28 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Leu	Leu	Phe	Leu	Leu	Ser	Thr	Tyr	Lys	Gln	Lys	Leu	Arg	Gln	Leu	Lys
1				5					10					15	
Lys	Asp	Gln	Lys	Lys	Leu	Glu	Gln	Leu	Pro	Thr	Ser				
				20				25							

(2) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 28 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS:
  - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Glu	Thr	Val	Asn	Val	Asn	Lys	Leu	Lys	Thr	Glu	Asn	Lys	Gln	Leu	Lys
1				5					10					15	
Lys	Glu	Val	Asp	Lys	Leu	Thr	Asn	Gly	Pro	Ala	Thr				
				20				25							

## (2) INFORMATION FOR SEQ ID NO: 25:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10443 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "plasmid"

## (iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GGCCGCCGCC ATGACGACGT CAAATGTAGA ATTGATACCA ATCTACACGG ATTGGGCCAA	60
TCGGCACCTT TCGAAGGGCA GCTTATCAAA GTCGATTAGG GATATTTCCA ATGATTTTCG	120
CGACTATCGA CTGGTTTCTC AGCTTATTAA TGTGATCGTT CCGATCAACG AATTCTCGCC	180
TGCATTACAG AAACGTTTGG CAAAAATCAC ATCGAACCTG GATGGCCTCG AAACGTGTCT	240
CGACTACCTG AAAAATCTGG GTCTCGACTG CTCGAACTC ACCAAAACCG ATATCGACAG	300
CGGAAACTTG GGTGCAGTTC TCCAGCTGCT CTTCTGCTC TCCACCTACA AGCAGAAGCT	360
TCGGCAACTG AAAAAAGATC AGAAGAAATT GGAGCAACTA CCCACATCCA TTATGCCACC	420
CGCGGTTTCT AAATTACCCT CGCCACGTGT CGCCACGTCA GCAACCGCTT CAGCAACTAA	480
CCCAAATTCC AACTTTCCAC AAATGTCAAC ATCCAGGCTT CAGACTCCAC AGTCAAGAAT	540
ATCGAAAATT GATTCATCAA AGATTGGTAT CAAGCCAAAG ACGTCTGGAC TTAAACCACC	600
CTCATCATCA ACCACTTCAT CAAATAATAC AAATTCATTC CGTCCGTCGA GCCGTTGAG	660
TGGCAATAAT AATGTTGGCT CGACGATATC CACATCTGCG AAGAGCTTAG AATCATCATC	720
AACGTACAGC TCTATTTTGA ATCTAAACCG ACCTACCTCC CAACTCCAAA AACCTTCTAG	780
ACCACAAACC CAGCTAGTTC GTGTTGCTAC AACTACAAA ATCGGAAGCT CAAAGCTAGC	840
CGCTCCGAAA GCCGTGAGCA CCCCCAACT TGCTTCTGTG AAGACTATTG GAGCAAAACA	900
AGAGCCCGAT AACAGCGGTG GTGGTGGTGG TGAATGCTG AAATTAAAGT TATTCAGTAG	960
CAAAAACCCA TCTTCCTCAT CGAATAGCCC ACAACCTACG AGAAAGGCGG CGGCGGTGCC	1020
TCAACAACAA ACTTTGTGCA AAATCGCTGC CCCAGTGAAA AGTGGCCTGA AGCCGCCGAC	1080
CAGTAAGCTG GGAAGTGCCA CGTCTATGTC GAAGCTTTGT ACGCCAAAAG TTTCTACCG	1140
TAAAACGGAC GCCCCAATCA TATCTCAACA AGACTCGAAA CGATGCTCAA AGAGCAGTGA	1200
AGAAGAGTCC GGATACGCTG GATTCAACAG CACGTCGCCA ACGTCATCAT CGACGGAAGG	1260
TTCCCTAAGC ATGCATTCCA CATCTTCCAA GAGTTCAACG TCAGACGAAA AGTCTCCGTC	1320
ATCAGACGAT CTTACTCTTA ACGCCTCCAT CGTGACAGCT ATCAGACAGC CGATAGCCGC	1380
AACACCGGTT TCTCCAAATA TTATCAACAA GCCTGTTGAG GAAAAACCAA CACTGGCAGT	1440



GAAAGGAGTG	AAAAGCACAG	CGAAAAAAGA	TCCACCTCCA	GCTGTTCCGC	CACGTGACAC	1500
CCAGCCAACA	ATCGGAGTTG	TTAGTCCAAT	TATGGCACAT	AAGAAGTTGA	CAAATGACCC	1560
CGTGATATCT	GAAAAACCAG	AACCTGAAAA	GCTCCAATCA	ATGAGCATCG	ACACGACGGA	1620
CGTTCCACCG	CTTCCACCTC	TAAAATCAGT	TGTTCCACTT	AAAATGACTT	CAATCCGACA	1680
ACCACCAACG	TACGATGTTT	TTCTAAAACA	AGGAAAAATC	ACATCGCCTG	TCAAGTCGTT	1740
TGGATATGAG	CAGTCGTCCG	CGTCTGAAGA	CTCCATTGTG	GCTCATGCGT	CGGCTCAGGT	1800
GACTCCGCCG	ACAAAAACTT	CTGGTAATCA	TTCGCTGGAG	AGAAGGATGG	GAAAGAATAA	1860
GACATCAGAA	TCCAGCGGCT	ACACCTCTGA	CGCCGGTGTT	GCGATGTGCG	CCAAAAATGAG	1920
GGAGAAGCTG	AAAGAATACG	ATGACATGAC	TCGTCGAGCA	CAGAACGGCT	ATCCTGACAA	1980
CTTCGAAGAC	AGTTCCTCCT	TGTCGTCTGG	AATATCCGAT	AACAACGAGC	TCGACGACAT	2040
ATCCACGGAC	GATTTGTCCG	GAGTAGACAT	GGCAACAGTC	GCCTCCAAAC	ATAGCGACTA	2100
TTCCCACTTT	GTTGCGCATC	CCACGTCTTC	TTCCTCAAAG	CCCCGAGTCC	CCAGTCGGTC	2160
CTCCACATCA	GTCGATTCTC	GATCTCGAGC	AGAACAGGAG	AATGTGTACA	AACTTCTGTC	2220
CCAGTGCCGA	ACGAGCCAAC	GTGGCGCCGC	TGCCACCTCA	ACCTTCGGAC	AACATTGCTT	2280
AAGATCCCCG	GGATACTCAT	CCTATTCTCC	ACACTTATCA	GTGTCAGCTG	ATAAGGACAC	2340
AATGTCTATG	CACTCACAGA	CTAGTCGACG	ACCTTCTTCA	CAAAAACCAA	GCTATTGAGG	2400
CCAATTTTCAT	TCACTTGATC	GTAAATGCCA	CCTTCAAGAG	TTCACATCCA	CCGAGCACAG	2460
AATGGCGGCT	CTCTTGAGCC	CGAGACGGGT	GCCGAAGTCG	ATGTCGAAAT	ATGATTCTTC	2520
AGGATCCTAC	TCGGCGCGTT	CCCGAGGTGG	AAGCTCTACT	GGTATCTATG	GAGAGACGTT	2580
CCAACTGCAC	AGACTATCCG	ATGAAAAATC	CCCCGCACAT	TCTGCCAAAA	GTGAGATGGG	2640
ATCCCAACTA	TCACTGGCTA	GCACGACAGC	ATATGGATCT	CTCAATGAGA	AGTACGAACA	2700
TGCTATTCCG	GACATGGCAC	GTGACTTGGA	GTGTTACAAG	AACACTGTCT	ACTCACTAAC	2760
CAAGAAACAG	GAGAACTATG	GAGCATTGTT	TGATCTTTTT	GAGCAAAAGC	TTAGAAAAGT	2820
CACTCAACAC	ATTGATCGAT	CCAACTTGAA	GCCTGAAGAG	GCAATACGAT	TCAGGCAGGA	2880
CATTGCTCAT	TTGAGGGATA	TTAGCAATCA	TCTTGATCC	AACTCAGCTC	ATGCTAACGA	2940
AGGCGCTGGT	GAGCTTCTTC	GTCAACCATC	TCTGGAATCA	GTTGCATCCC	ATCGATCATC	3000
GATGTCATCG	TCGTCGAAAA	GCAGCAAGCA	GGAGAAGATC	AGCTTGAGCT	CGTTTGCCAA	3060
GAACAAGAAG	AGCTGGATCC	GCTCCTCACT	CTCCAAGTTC	ACCAAGAAGA	AGAACAAGAA	3120
CTACGACGAA	GCACATATGC	CATCAATTTT	CGGATCTCAA	GGAAGTCTTG	ACAACATTGA	3180
TGTGATTGAG	TTGAAGCAAG	AGCTCAAAGA	ACGCGATAGT	GCACTTTACG	AAGTCCGCTT	3240
TGACAATCTG	GATCGTGCCC	GCGAAGTTGA	TGTTCTGAGG	GAGACAGTGA	ACAAGTTGAA	3300
AACCGAGAAC	AAGCAATTAA	AGAAAGAAGT	GGACAAACTC	ACCAACGGTC	CAGCCACTCG	3360

TGCTTCTTCC	CGCGCCTCAA	TTCCAGTTAT	CTACGACGAT	GAGCATGTCT	ATGATGCAGC	3420
GTGTAGCAGT	ACATCAGCTA	GTCAATCTTC	GAAACGATCC	TCTGGCTGCA	ACTCAATCAA	3480
GGTTACTGTA	AACGTGGACA	TCGCTGGAGA	AATCAGTTTCG	ATCGTTAACC	CGGACAAAGA	3540
GATAATCGTA	GGATATCTTG	CCATGTCAAC	CAGTCAGTCA	TGCTGGAAAG	ACATTGATGT	3600
TTCTATTCTA	GGACTATTTG	AAGTCTACCT	ATCCAGAATT	GATGTGGAGC	ATCAACTTGG	3660
AATCGATGCT	CGTGATTCTA	TCCTTGGCTA	TCAAATTGGT	GAACTTCGAC	GCGTCATTGG	3720
AGACTCCACA	ACCATGATAA	CCAGCCATCC	AACTGACATT	CTTACTTCCT	CAACTACAAT	3780
CCGAATGTTT	ATGCACGGTG	CCGCACAGAG	TCGCGTAGAC	AGTCTGGTCC	TTGATATGCT	3840
TCTTCCAAAG	CAAATGATTC	TCCAACCTCGT	CAAGTCAATT	TTGACAGAGA	GACGTCTGGT	3900
GTTAGCTGGA	GCAACTGGAA	TTGGAAAGAG	CAAACTGGCG	AAGACCCTGG	CTGCTTATGT	3960
ATCTATTCTA	ACAAATCAAT	CCGAAGATAG	TATTGTTAAT	ATCAGCATTG	CTGAAAACAA	4020
TAAAGAAGAA	TTGCTTCAAG	TGGAACGACG	CCTGGAAAAG	ATCTTGAGAA	GCAAAGAATC	4080
ATGCATCGTA	ATTCTAGATA	ATATCCCAAA	GAATCGAATT	GCATTTGTTG	TATCCGTTTT	4140
TGCAAATGTC	CCACTTCAAA	ACAACGAAGG	TCCATTTGTA	GTATGCACAG	TCAACCGATA	4200
TCAAATCCCT	GAGCTTCAAA	TTCACCACAA	TTTCAAAATG	TCAGTAATGT	CGAATCGTCT	4260
CGAAGGATTC	ATCCTACGTT	ACCTCCGACG	ACGGGCGGTA	GAGGATGAGT	ATCGTCTAAC	4320
TGTACAGATG	CCATCAGAGC	TCTTCAAAAT	CATTGACTTC	TTCCCAATAG	CTCTTCAGGC	4380
CGTCAATAAT	TTTATTGAGA	AAACGAATTC	TGTTGATGTG	ACAGTTGGTC	CAAGAGCATG	4440
CTTGAAGTGT	CCTCTAACTG	TCGATGGATC	CCGTGAATGG	TTCATTCGAT	TGTGGAATGA	4500
GAACTTCATT	CCATATTTGG	AACGTGTTGC	TAGAGATGGC	AAAAAAACCT	TCGGTCGCTG	4560
CACTTCCTTC	GAGGATCCCA	CCGACATCGT	CTCTAAAAAA	TGGCCGTGGT	TCGATGGTGA	4620
AAACCCGGAG	AATGTGCTCA	AACGTCTTCA	ACTCCAAGAC	CTCGTCCCGT	CACCTGCCAA	4680
CTCATCCCGA	CAACACTTCA	ATCCCCTCGA	GTCGTTGATC	CAATTGCATG	CTACCAAGCA	4740
TCAGACCATC	GACAACATTT	GAACAGAAGA	CTCTAATCTT	CTCTCGCCTC	TCCCCCGCTT	4800
TCCTTATCTT	CGTACCGGTA	CCTGATGATT	CCCCATTTTC	CCCCTTTTCC	CCCCAATTTT	4860
CCAGAACCTC	CTGTTCCCTT	TGTTCCTAGT	CCTCCCGGGT	GCCGACGCCG	AAGCGATTTA	4920
AAAACCTTTT	TCTTTCCGAA	ACATTTCCCA	TTGCTCATT	ATAGTCAAAT	TGAATAAACA	4980
GTGTATGTAC	TTAAAAAAA	AAAAAAA	ACTCGAGGGG	GGGCCCTATT	CTATAGTGTC	5040
ACCTAAATGC	TAGAGCTCGC	TGATCAGCCT	CGACTGTGCC	TTCTAGTTGC	CAGCCATCTG	5100
TTGTTTGCCC	CTCCCCCGTG	CCTTCCTTGA	CCCTGGAAGG	TGCCACTCCC	ACTGTCCTTT	5160
CCTAATAAAA	TGAGGAAATT	GCATCGCATT	GTCTGAGTAG	GTGTCATTCT	ATTCTGGGGG	5220
GTGGGGTGGG	GCAGGACAGC	AAGGGGGAGG	ATTGGGAAGA	CAATAGCAGG	CATGCTGGGG	5280

ATGCGGTGGG CTCTATGGCT TCTGAGGCGG AAAGAACCAG CTGGGGCTCT AGGGGGTATC	5340
CCCACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG CGCAGCGTGA	5400
CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTTCGC TTTCTTCCCT TCCTTTCTCG	5460
CCACGTTTCGC CGGCTTTCCC CGTCAAGCTC TAAATCGGGG CATCCCTTTA GGGTTCGGAT	5520
TTAGTGCTTT ACGGCACCTC GACCCCAAAA AACTTGATTA GGGTGATGGT TCACGTAGTG	5580
GGCCATCGCC CTGATAGACG GTTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA	5640
GTGGACTCTT GTTCCAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT TCTTTTGATT	5700
TATAAGGGAT TTTGGGGATT TCGGCCTATT GGTTAAAAAA TGAGCTGATT TAACAAAAAT	5760
TTAACGCGAA TTAATTCTGT GGAATGTGTG TCAGTTAGGG TGTGGAAAGT CCCCAGGCTC	5820
CCCAGGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC AGGTGTGGAA	5880
AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA	5940
CCATAGTCCC GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCAT	6000
CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC GCCTCTGCCT	6060
CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTGGAGG CCTAGGCTTT TGCAAAAAGC	6120
TCCCGGGAGC TTGTATATCC ATTTTCGGAT CTGATCAAGA GACAGGATGA GGATCGTTTC	6180
GCATGATTGA ACAAGATGGA TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT	6240
TCGGCTATGA CTGGGCACAA CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT	6300
CAGCGCAGGG GCGCCCGGTT CTTTTTGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC	6360
TGCAGGACGA GGCAGCGCGG CTATCGTGGC TGGCCACGAC GGGCGTTCCT TGCAGCTG	6420
TGCTCGACGT TGTCAGTAA GCGGGAAGGG ACTGGCTGCT ATTGGGCGAA GTGCCGGGGC	6480
AGGATCTCCT GTCATCTCAC CTTGCTCCTG CCGAGAAAGT ATCCATCATG GCTGATGCAA	6540
TGCGGCGGCT GCATACGCTT GATCCGGCTA CCTGCCCAT TCGACCACCA GCGAAACATC	6600
GCATCGAGCG AGCAGCTACT CGGATGGAAG CCGGTCTTGT CGATCAGGAT GATCTGGACG	6660
AAGAGCATCA GGGGCTCGCG CCAGCCGAAC TGTTCCGCCAG GCTCAAGGCG CGCATGCCCCG	6720
ACGGCGAGGA TCTCGTCGTG ACCCATGGCG ATGCCTGCTT GCCGAATATC ATGGTGGA	6780
ATGGCCGCTT TTCTGGATT CACTGACTGTG GCCGGCTGGG TGTGGCGGAC CGCTATCAGG	6840
ACATAGCGTT GGCTACCCGT GATATTGCTG AAGAGCTTGG CGGCGAATGG GCTGACCGCT	6900
TCCTCGTGCT TTACGGTATC GCCGCTCCCG ATTCGCAGCG CATCGCCTTC TATCGCCTTC	6960
TTGACGAGTT CTTCTGAGCG GGACTCTGGG GTTCGAAATG ACCGACCAAG CGACGCCCAA	7020
CCTGCCATCA CGAGATTTCG ATTCCACCGC CGCCTTCTAT GAAAGGTTGG GCTTCGGAAT	7080
CGTTTTCCGG GACGCCGGCT GGATGATCCT CCAGCGCGGG GATCTCATGC TGGAGTTCTT	7140
CGCCCACCCC AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC	7200

AAATTTTACACA	AATAAAGCAT	TTTTTTCACCT	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	7260
CAATGTATCT	TATCATGTCT	GTATACCGTC	GACCTCTAGC	TAGAGCTTGG	CGTAATCATG	7320
GTCATAGCTG	TTTCCTGTGT	GAAATTGTTA	TCCGCTCACA	ATTCCACACA	ACATACGAGC	7380
CGGAAGCATA	AAGTGTAAG	CCTGGGGTGC	CTAATGAGTG	AGCTAACTCA	CATTAATTGC	7440
GTTGCGCTCA	CTGCCCCTT	TCCAGTCGGG	AAACCTGTCG	TGCCAGCTGC	ATTAATGAAT	7500
CGGCCAACGC	GCGGGGAGAG	GCGGTTTGCG	TATTGGGCGC	TCTTCCGCTT	CCTCGCTCAC	7560
TGACTCGCTG	CGCTCGGTG	TTCGGCTGCG	GCGAGCGGTA	TCAGCTCACT	CAAAGGCGGT	7620
AATACGGTTA	TCCACAGAAT	CAGGGGATAA	CGCAGGAAAG	AACATGTGAG	CAAAAGGCCA	7680
GCAAAAGGCC	AGGAACCGTA	AAAAGGCCGC	GTTGCTGGCG	TTTTTCCATA	GGCTCCGCCC	7740
CCCTGACGAG	CATCACAAAA	ATCGACGCTC	AAGTCAGAGG	TGGCGAAACC	CGACAGGACT	7800
ATAAAGATAC	CAGGCGTTTC	CCCCTGGAAG	CTCCCTCGTG	CGCTCTCCTG	TTCCGACCCT	7860
GCCGCTTACC	GGATACCTGT	CCGCCTTTCT	CCCTTCGGGA	AGCGTGCGC	TTTCTCAATG	7920
CTCACGCTGT	AGGTATCTCA	GTTGCGGTGA	GGTCGTTTCG	TCCAAGCTGG	GCTGTGTGCA	7980
CGAACCCCC	GTTACGCCCC	ACCGCTGCGC	CTTATCCGGT	AACTATCGTC	TTGAGTCCAA	8040
CCCGGTAAGA	CACGACTTAT	CGCCACTGGC	AGCAGCCACT	GGTAACAGGA	TTAGCAGAGC	8100
GAGGTATGTA	GGCGGTGCTA	CAGAGTTCTT	GAAGTGGTGG	CCTAACTACG	GCTACACTAG	8160
AAGGACAGTA	TTTGGTATCT	GCGCTCTGCT	GAAGCCAGTT	ACCTTCGGAA	AAAGAGTTGG	8220
TAGCTCTTGA	TCCGGCAAAC	AAACCACCGC	TGGTAGCGGT	GGTTTTTTTG	TTTGCAAGCA	8280
GCAGATTACG	CGCAGAAAAA	AAGGATCTCA	AGAAGATCCT	TTGATCTTTT	CTACGGGGTC	8340
TGACGCTCAG	TGGAACGAAA	ACTCACGTTA	AGGGATTTTG	GTCATGAGAT	TATCAAAAAG	8400
GATCTTCACC	TAGATCCTTT	TAAATTAAAA	ATGAAGTTTT	AAATCAATCT	AAAGTATATA	8460
TGAGTAAACT	TGGTCTGACA	GTTACCAATG	CTTAATCAGT	GAGGCACCTA	TCTCAGCGAT	8520
CTGTCTATTT	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC	GTGTAGATAA	CTACGATACG	8580
GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC	AATGATACCG	CGAGACCCAC	GCTCACCGGC	8640
TCCAGATTTA	TCAGCAATAA	ACCAGCCAGC	CGGAAGGGCC	GAGCGCAGAA	GTGGTCCTGC	8700
AACTTTATCC	GCCTCCATCC	AGTCTATTAA	TTGTTGCCGG	GAAGCTAGAG	TAAGTAGTTC	8760
GCCAGTTAAT	AGTTTGCGCA	ACGTTGTTGC	CATTGCTACA	GGCATCGTGG	TGTCACGCTC	8820
GTCGTTTGGT	ATGGCTTCAT	TCAGCTCCGG	TTCCCAACGA	TCAAGGCGAG	TTACATGATC	8880
CCCCATGTTG	TGCAAAAAAG	CGGTTAGCTC	CTTCGGTCTT	CCGATCGTTG	TCAGAAGTAA	8940
GTTGGCCGCA	GTGTTATCAC	TCATGGTTAT	GGCAGCACTG	CATAATTCTC	TTACTGTGAT	9000
GCCATCCGTA	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA	ACCAAGTCAT	TCTGAGAATA	9060
GTGTATGCGG	CGACCGAGTT	GCTCTTGCCC	GGCGTCAATA	CGGGATAATA	CCGCGCCACA	9120

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TAGCAGAACT TTAAGAGTGC TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG	9180
GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGACCCCA ACTGATCTTC	9240
AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC	9300
AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTCAATA	9360
TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA	9420
GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC CTGACGTCGA	9480
CGGATCGGGA GATCTCCCGA TCCCCTATGG TCGACTCTCA GTACAATCTG CTCTGATGCC	9540
GCATAGTTAA GCCAGTATCT GCTCCCTGCT TGTGTGTTGG AGGTCGCTGA GTAGTGCGCG	9600
AGCAAAATTT AAGCTACAAC AAGGCAAGGC TTGACCGACA ATTGCATGAA GAATCTGCTT	9660
AGGGTTAGGC GTTTTGCGCT GCTTCGCGAT GTACGGGCCA GATATACGCG TTGACATTGA	9720
TTATTGACTA GTTATTAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG CCCATATATG	9780
GAGTTCCGCG TTACATAACT TACGGTAAAT GGCCCGCCTG GCTGACCGCC CAACGACCCC	9840
CGCCCATGTA CGTCAATAAT GACGTATGTT CCCATAGTAA CGCCAATAGG GACTTTCCAT	9900
TGACGTCAAT GGGTGGACTA TTTACGGTAA ACTGCCCACT TGGCAGTACA TCAAGTGAT	9960
CATATGCCAA GTACGCCCC TATTGACGTC AATGACGGTA AATGGCCCCG CTGGCATTAT	10020
GCCCAGTACA TGACCTTATG GGACTTTCCT ACTTGGCAGT ACATCTACGT ATTAGTCATC	10080
GCTATTACCA TGGTGATGCG GTTTTGGCAG TACATCAATG GGCGTGGATA GCGGTTTGAC	10140
TCACGGGGAT TTCCAAGTCT CCACCCCAT TACGTCAATG GGAGTTTGTT TTGGCACCAA	10200
AATCAACGGG ACTTTCCAA ATGTCGTAAC AACTCCGCCC CATTGACGCA AATGGGCGGT	10260
AGGCGTGTAC GGTGGGAGGT CTATATAAGC AGAGCTCTCT GGCTAACTAG AGAACCCACT	10320
GCTTACTGGC TTATCGAAAT TAATACGACT CACTATAGGG AGACCCAAGC TTGGTACCGA	10380
GCTCGGATCC ACTAGTAACG GCCGCCAGTG TGCTGGAATT CTGCAGATAT CCATCACACT	10440
GGC	10443

## (2) INFORMATION FOR SEQ ID NO: 26:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7474 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "plasmid"

## (iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CTAAATTGTA AGCGTTAATA TTTTGTAAAA ATTCGCGTTA AATTTTTGTT AAATCAGCTC	60
ATTTTTTAAC CAATAGGCCG AAATCGGCAA AATCCCTTAT AAATCAAAAG AATAGACCGA	120
GATAGGGTTG AGTGTGTGTC CAGTTTGGA CAAGAGTCCA CTATTAAAGA ACGTGGACTC	180
CAACGTCAAA GGGCGAAAAA CCGTCTATCA GGGCGATGGC CCACTACGTG AACCATCACC	240
CTAATCAAGT TTTTGGGGT CGAGGTGCCG TAAAGCACTA AATCGGAACC CTAAAGGGAG	300
CCCCGATTT AGAGCTTGAC GGGGAAAGCC GGCGAACGTG GCGAGAAAGG AAGGGAAGAA	360
AGCGAAAGGA GCGGGCGCTA GGGCGCTGGC AAGTG TAGCG GTCACGCTGC GCGTAACCAC	420
CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTCC CATTCGCCAT TCAGGCTGCG	480
CAACTGTTGG GAAGGGCGAT CGGTGCGGGC CTCTTCGCTA TTACGCCAGC TGGCGAAAGG	540
GGGATGTGCT GCAAGGCGAT TAAGTTGGGT AACGCCAGGG TTTTCCCAGT CACGACGTTG	600
TAAACGACG GCCAGTGAGC GCGCGTAATA CGACTCACTA TAGGGCGAAT TGGAGCTCCA	660
CCGCGGTTTC TAAATTACCC TCGCCACGTG TCGCCACGTC AGCAACCGCT TCAGCAACTA	720
ACCCAAATTC CAACTTTCCA CAAATGTCAA CATCCAGGCT TCAGACTCCA CAGTCAAGAA	780
TATCGAAAAT TGATTCATCA AAGATTGGTA TCAAGCCAAA GACGTCTGGA CTTAAACCAC	840
CCTCATCATC AACCCTTCA TCAAATAATA CAAATTCATT CCGTCCGTCG AGCCGTTCTGA	900
GTGGCAATAA TAATGTTGGC TCGACGATAT CCACATCTGC GAAGAGCTTA GAATCATCAT	960
CAACGTACAG CTCTATTTG AATCTAAACC GACCTACCTC CCAACTCCAA AAACCTTCTA	1020
GACCACAAAC CCAGCTAGTT CGTGTGCTA CAACTACAAA AATCGGAAGC TCAAAGCTAG	1080
CCGCTCCGAA AGCCGTGAGC ACCCCAAAAC TTGCTTCTGT GAAGACTATT GGAGCAAAAC	1140
AAGAGCCCGA TAACAGCGGT GGTGGTGGTG GTGGAATGCT GAAATTAAAG TTATTGAGTA	1200
GCAAAACCC ATCTTCCTCA TCGAATAGCC CACAACCTAC GAGAAAGGCG GCGGCGGTGC	1260
CTCAACAACA AACTTTGTG AAAATCGCTG CCCAGTGAA AAGTGGCCTG AAGCCGCCGA	1320
CCAGTAAGCT GGGAAAGTGCC ACGTCTATGT CGAAGCTTTG TACGCCAAAA GTTTCCTACC	1380
GTAAAACGGA CGCCCCAATC ATATCTCAAC AAGACTCGAA ACGATGCTCA AAGAGCAGTG	1440
AAGAAGAGTC CGGATACGCT GGATTCAACA GCACGTCGCC AACGTCATCA TCGACGGAAG	1500
GTTCCCTAAG CATGCATTCC ACATCTTCCA AGAGTTCAAC GTCAGACGAA AAGTCTCCGT	1560
CATCAGACGA TCTTACTCTT AACGCCTCCA TCGTGACAGC TATCAGACAG CCGATAGCCG	1620
CAACACCGGT TTCTCCAAAT ATTATCAACA AGCCTGTTGA GGAAAAACCA AACTGGCAG	1680
TGAAAGGAGT GAAAAGCACA GCGAAAAAG ATCCACCTCC AGCTGTTCCG CCACGTGACA	1740
CCCAGCCAAC AATCGGAGTT GTTAGTCCAA TTATGGCACA TAAGAAGTTG ACAAATGACC	1800
CCGTGATATC TGAAAAACCA GAACCTGAAA AGCTCCAATC AATGAGCATC GACACGACGG	1860

ACGTTCCACC	GCTTCCACCT	CTAAAATCAG	TTGTTCCACT	TAAAATGACT	TCAATCCGAC	1920
AACCACCAAC	GTACGATGTT	CTTCTAAAAC	AAGGAAAAAT	CACATCGCCT	GTCAAGTCGT	1980
TTGGATATGA	GCAGTCGTCC	GCGTCTGAAG	ACTCCATTGT	GGCTCATGCG	TCGGCTCAGG	2040
TGACTCCGCC	GACAAAAACT	TCTGGTAATC	ATTCGCTGGA	GAGAAGGATG	GGAAAGAATA	2100
AGACATCAGA	ATCCAGCGGC	TACACCTCTG	ACGCCGGTGT	TGCGATGTGC	GCCAAAATGA	2160
GGGAGAAGCT	GAAAGAATAC	GATGACATGA	CTCGTCGAGC	ACAGAACGGC	TATCCTGACA	2220
ACTTCGAAGA	CAGTTCCTCC	TTGTCTCTG	GAATATCCGA	TAACAACGAG	CTCGACGACA	2280
TATCCACGGA	CGATTTGTCC	GGAGTAGACA	TGGCAACAGT	CGCCTCCAAA	CATAGCGACT	2340
ATTCCCACTT	TGTTCCGCAT	CCCACGTCTT	CTTCCTCAA	GCCCCGAGTC	CCCAGTCGGT	2400
CCTCCACATC	AGTCGATTCT	CGATCTCGAG	CAGAACAGGA	GAATGTGTAC	AAACTTCTGT	2460
CCCAGTGCCG	AACGAGCCAA	CGTGGCGCCG	CTGCCACCTC	AACCTTCGGA	CAACATTCGC	2520
TAAGATCCCC	GGGATACTCA	TCCTATTCTC	CACACTTATC	AGTGTGAGCT	GATAAGGACA	2580
CAATGTCTAT	GCACTCACAG	ACTAGTCGAC	GACCTTCTTC	ACAAAAACCA	AGCTATTCAG	2640
GCCAATTTCA	TTCACTTGAT	CGTAAATGCC	ACCTTCAAGA	GTTACATCC	ACCGAGCACA	2700
GAATGGCGGC	TCTCTTGAGC	CCGAGACGGG	TGCCGAATC	GATGTCGAAA	TATGATTCTT	2760
CAGGATCCTA	CTCGGCGCGT	TCCCGAGGTG	GAAGCTCTAC	TGGTATCTAT	GGAGAGACGT	2820
TCCAAC TGCA	CAGACTATCC	GATGAAAAAT	CCCCCGCACA	TTCTGCCAAA	AGTGAGATGG	2880
GATCCCAACT	ATCACTGGCT	AGCACGACAG	CATATGGATC	TCTCAATGAG	AAGTACGAAC	2940
ATGCTATTCTG	GGACATGGCA	CGTGACTTGG	AGTGTTACAA	GAACACTGTC	GACTCACTAA	3000
CCAAGAAACA	GGAGAACTAT	GGAGCATTGT	TTGATCTTTT	TGAGCAAAG	CTTAGAAAAC	3060
TCACTCAACA	CATTGATCGA	TCCAAC TTGA	AGCCTGAAGA	GGCAATACGA	TTCAGGCAGG	3120
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AAGGCGCTGG	TGAGCTTCTT	CGTCAACCAT	CTCTGGAATC	AGTTGCATCC	CATCGATCAT	3240
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ATGTGATTGA	GTTGAAGCAA	GAGCTCAAAG	AACGCGATAG	TGCACTTTAC	GAAGTCCGCC	3480
TTGACAATCT	GGATCGTGCC	CGCGAAGTTG	ATGTTCTGAG	GGAGACAGTG	AACAAGTTGA	3540
AAACCGAGAA	CAAGCAATTA	AAGAAAGAAG	TGGACAAACT	CACCAACGGT	CCAGCCACTC	3600
GTGCTTCTTC	CCGCGCCTCA	ATTCCAGTTA	TCTACGACGA	TGAGCATGTC	TATGATGCAG	3660
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CATGCATCGT	AATTCTAGAT	AATATCCCAA	AGAATCGAAT	TGCATTTGTT	GTATCCGTTT	4380
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AAAAACCTTT	TTCTTTCCGA	AACATTTCCC	ATTGCTCATT	AATAGTCAAA	TTGAATAAAC	5220
AGTGTATGTA	CTTAAAAAAA	AAAAAAAAAA	AACCTGAGGG	GGGGCCCGGT	ACCCAGCTTT	5280
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GCTTTCCAGT	CGGGAAACCT	GTCGTGCCAG	CTGCATTAAT	GAATCGGCCA	ACGCGCGGGG	5520
AGAGGCGGTT	TGCGTATTGG	GCGCTCTTCC	GCTTCTCTCG	TCACTGACTC	GCTGCGCTCG	5580
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CTCAGTTCGG	TGTAGGTCGT	TCGCTCCAAG	CTGGGCTGTG	TGCACGAACC	CCCCGTTGAG	6000
CCCGACCGCT	GCGCCTTATC	CGGTA ACTAT	CGTCTTGAGT	CCAACCCGGT	AAGACACGAC	6060
TTATCGCCAC	TGGCAGCAGC	CACTGGTAAC	AGGATTAGCA	GAGCGAGGTA	TGTAGGCGGT	6120
GCTACAGAGT	TCTTGAAGTG	GTGGCCTAAC	TACGGCTACA	CTAGAAGGAC	AGTATTTGGT	6180
ATCTGCGCTC	TGCTGAAGCC	AGTTACCTTC	GGAAAAAGAG	TTGGTAGCTC	TTGATCCGGC	6240
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AAAAAAGGAT	CTCAAGAAGA	TCCTTTGATC	TTTTCTACGG	GGTCTGACGC	TCAGTGGAAC	6360
GAAAACTCAC	GTTAAGGGAT	TTTGGTCATG	AGATTATCAA	AAAGGATCTT	CACCTAGATC	6420
CTTTTAAATT	AAAAATGAAG	TTTTAAATCA	ATCTAAAGTA	TATATGAGTA	AACTTGGTCT	6480
GACAGTTACC	AATGCTTAAT	CAGTGAGGCA	CCTATCTCAG	CGATCTGTCT	ATTCGTTTCA	6540
TCCATAGTTG	CCTGACTCCC	CGTCGTGTAG	ATAACTACGA	TACGGGAGGG	CTTACCATCT	6600
GGCCCCAGTG	CTGCAATGAT	ACCGCGAGAC	CCACGCTCAC	CGGCTCCAGA	TTTATCAGCA	6660
ATAAACCCAGC	CAGCCGGAAG	GGCCGAGCGC	AGAAGTGGTC	CTGCAACTTT	ATCCGCCTCC	6720
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TCACTCATGG	TTATGGCAGC	ACTGCATAAT	TCTCTTACTG	TCATGCCATC	CGTAAGATGC	7020
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AGATCCAGTT	CGATGTAACC	CACTCGTGCA	CCCAACTGAT	CTTCAGCATC	TTTTACTTTC	7260
ACCAGCGTTT	CTGGGTGAGC	AAAAACAGGA	AGGCAAAATG	CCGCAAAAAA	GGGAATAAGG	7320
GCGACACGGA	AATGTTGAAT	ACTCATACTC	TTCCTTTTTTC	AATATTATTG	AAGCATTTAT	7380
CAGGGTTATT	GTCTCATGAG	CGGATACATA	TTTGAATGTA	TTTAGAAAAA	TAAACAAATA	7440
GGGGTTCCGC	GCACATTTCC	CCGAAAAGTG	CCAC			7474

## (2) INFORMATION FOR SEQ ID NO: 27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13414 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "plasmid"

## (iii) HYPOTHETICAL: NO

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 11582
- (D) OTHER INFORMATION: /note= "N is A,G,C or T"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TATGACGACG TCAAATGTAG AATTGATACC ATTCTACACG GATTGGGCCA ATCGGCACCT	60
TTCGAAGGGC AGCTTATCAA AGTCGATTAG GGATATTTCC AATGATTTTC GCGACTATCG	120
ACTGGTTTCT CAGCTTATTA ATGTGATCGT TCCGATCAAC GAATTCTCGC CTGCATTACG	180
GAAACGTTTG GCAAAAATCA CATCGAACCT GGATGGCCTC GAAACGTGTC TCGACTACCT	240
GAAAAATCTG GGTCTCGACT GCTCGAACT CACCAAAACC GATATCGACA GCGGAAACTT	300
GGGTGCAGTT CTCCAGCTGC TCTTCCTGCT CTCCACCTAC AAGCAGAAGC TTCGGCAACT	360
GAAAAAAGAT CAGAAGAAAT TGGAGCAACT ACCCACATCC ATTATGCCAC CCGCGGTTTC	420
TAAATTACCC TCGCCACGTG TCGCCACGTC AGCAACCGCT TCAGCAACTA ACCCAAATTC	480
CAACTTTCCA CAAATGTCAA CATCCAGGCT TCAGACTCCA CAGTCAAGAA TATCGAAAAT	540
TGATTCATCA AAGATTGGTA TCAAGCCAAA GACGTCTGGA CTTAAACCAC CCTCATCATC	600
AACCACTTCA TCAAATAATA CAAATTCATT CCGTCCGTCG AGCCGTTCTGA GTGGCAATAA	660
TAATGTTGGC TCGACGATAT CCACATCTGC GAAGAGCTTA GAATCATCAT CAACGTACAG	720
CTCTATTTCTG AATCTAAACC GACCTACCTC CCAACTCCAA AAACCTTCTA GACCACAAAC	780
CCAGCTAGTT CGTGTGCTA CAACTACAAA AATCGGAAGC TCAAAGCTAG CCGCTCCGAA	840
AGCCGTGAGC ACCCCAAAAC TTGCTTCTGT GAAGACTATT GGAGCAAAAC AAGAGCCCGA	900
TAACAGCGGT GGTGGTGGTG GTGGAATGCT GAAATTAAAG TTATTCTAGTA GCAAAAACCC	960
ATCTTCCTCA TCGAATAGCC CACAACCTAC GAGAAAGGCG GCGGCGGTGC CTCAACAACA	1020
AACCTTGTCTG AAAATCGCTG CCCCAGTGAA AAGTGGCCTG AAGCCGCCGA CCAGTAAGCT	1080
GGGAAGTGCC ACGTCTATGT CGAAGCTTTG TACGCCAAAA GTTTCCTACC GTAAAACGGA	1140
CGCCCCAATC ATATCTCAAC AAGACTCGAA ACGATGCTCA AAGAGCAGTG AAGAAGAGTC	1200
CGGATACGCT GGATTCAACA GCACGTCGCC AACGTCATCA TCGACGGAAG GTTCCCTAAG	1260

CATGCATTCC	ACATCTTCCA	AGAGTTCAAC	GTCAGACGAA	AAGTCTCCGT	CATCAGACGA	1320
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GACAAAAACT	TCTGGTAATC	ATTGCTGGA	GAGAAGGATG	GGAAAGAATA	AGACATCAGA	1860
ATCCAGCGGC	TACACCTCTG	ACGCCGGTGT	TGCGATGTGC	GCCAAAATGA	GGGAGAAGCT	1920
GAAAGAATAC	GATGACATGA	CTCGTCGAGC	ACAGAACGGC	TATCCTGACA	ACTTCGAAGA	1980
CAGTTCCTCC	TTGTCGTCTG	GAATATCCGA	TAACAACGAG	CTCGACGACA	TATCCACGGA	2040
CGATTTGTCC	GGAGTAGACA	TGGCAACAGT	CGCCTCCAAA	CATAGCGACT	ATTCCCACCTT	2100
TGTTCGCCAT	CCCACGTCTT	CTTCCTCAAA	GCCCCGAGTC	CCCAGTCGGT	CCTCCACATC	2160
AGTCGATTCT	CGATCTCGAG	CAGAACAGGA	GAATGTGTAC	AACTTCTGT	CCCAGTGCCG	2220
AACGAGCCAA	CGTGGCGCCG	CTGCCACCTC	AACCTTCGGA	CAACATTTCG	TAAGATCCCC	2280
GGGATACTCA	TCCTATTCTC	CACACTTATC	AGTGTGAGCT	GATAAGGACA	CAATGTCTAT	2340
GCACTCACAG	ACTAGTCGAC	GACCTTCTTC	ACAAAAACCA	AGCTATTTCAG	GCCAATTTCA	2400
TTCATTGAT	CGTAAATGCC	ACCTTCAAGA	GTTACATCC	ACCGAGCACA	GAATGGCGGC	2460
TCTCTTGAGC	CCGAGACGGG	TGCCGAATC	GATGTCGAAA	TATGATTCTT	CAGGATCCTA	2520
CTCGGCGCGT	TCCCGAGGTG	GAAGCTCTAC	TGGTATCTAT	GGAGAGACGT	TCCAAC TGCA	2580
CAGACTATCC	GATGAAAAAT	CCCCCGCACA	TTCTGCCAAA	AGTGAGATGG	GATCCCAACT	2640
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GGACATGGCA	CGTGACTTGG	AGTGTTACAA	GAACACTGTC	GACTCACTAA	CCAAGAAACA	2760
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TGAGCTTCTT	CGTCAACCAT	CTCTGGAATC	AGTTGCATCC	CATCGATCAT	CGATGTCATC	3000
GTCGTCGAAA	AGCAGCAAGC	AGGAGAAGAT	CAGCTTGAGC	TCGTTTGGCA	AGAACAAGAA	3060
GAGCTGGATC	CGCTCCTCAC	TCTCCAAGTT	CACCAAGAAG	AAGAACAAGA	ACTACGACGA	3120
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GCAAATGATT	CTCCAAC TCG	TCAAGTCAAT	TTTGACAGAG	AGACGTCTGG	TGTTAGCTGG	3900
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CATCCTACGT	TACCTCCGAC	GACGGGCGGT	AGAGGATGAG	TATCGTCTAA	CTGTACAGAT	4320
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ACAACACTTC	AATCCCCTCG	AGTCGTTGAT	CCAATTGCAT	GCTACCAAGC	ATCAGACCAT	4740
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ACTACGATAC	GGGAGGGCTT	ACCATCTGGC	CCCAGTGCTG	CAATGATACC	GCGAGACCCA	6600
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## (2) INFORMATION FOR SEQ ID NO: 28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10288 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "plasmid"

## (iii) HYPOTHETICAL: NO

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 8456
- (D) OTHER INFORMATION: /note= "N is A,C,G, or T"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

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TGCCCGCGAA GTTGATGTTT TGAGGGAGAC AGTGAACAAG TTGAAAACCG AGAACAAGCA 180
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ATTTGAAGTC TACCTATCCA GAATTGATGT GGAGCATCAA CTTGGAATCG ATGCTCGTGA 540

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GAAAACGAGC	GCTTCCGAAA	ATGCAACGCG	AGCTGCGCAC	ATACAGCTCA	CTGTTCACGT	5400
CGCACCTATA	TCTGCGTGTT	GCCTGTATAT	ATATATACAT	GAGAAGAACG	GCATAGTGCG	5460
TGTTTATGCT	TAAATGCGTA	CTTATATGCG	TCTATTTATG	TAGGATGAAA	GGTAGTCTAG	5520
TACCTCCTGT	GATATTATCC	CATTCCATGC	GGGGTATCGT	ATGCTTCCTT	CAGCACTACC	5580
CTTTAGCTGT	TCTATATGCT	GCCACTCCTC	AATTGGATTA	GTCTCATCCT	TCAATGCTAT	5640
CATTTCCCTT	GATATTGGAT	CATATTAAGA	AACCATTATT	ATCATGACAT	TAACCTATAA	5700
AAATAGGCGT	ATCACGAGGC	CCTTTCGTCT	CGCGCGTTTC	GGTGATGACG	GTGAAAACCT	5760
CTGACACATG	CAGCTCCCGG	AGACGGTCAC	AGCTTGTCTG	TAAGCGGATG	CCGGGAGCAG	5820
ACAAGCCCGT	CAGGGCGCGT	CAGCGGGTGT	TGGCGGGTGT	CGGGGCTGGC	TTAACTATGC	5880
GGCATCAGAG	CAGATTGTAC	TGAGAGTGCA	CCATAGATCA	ACGACATTAC	TATATATATA	5940
ATATAGGAAG	CATTTAATAG	ACAGCATCGT	AATATATGTG	TACTTTGCAG	TTATGACGCC	6000
AGATGGCAGT	AGTGGAAAGT	ATTCTTTATT	GAAAAATAGC	TTGTCACCTT	ACGTACAATC	6060
TTGATCCGGA	GCTTTTCTTT	TTTTGCCGAT	TAAGAATTAA	TTCGGTCGAA	AAAAGAAAAG	6120
GAGAGGGCCA	AGAGGGAGGG	CATTGGTGAC	TATTGAGCAC	GTGAGTATAC	GTGATTAAGC	6180
ACACAAAGGC	AGCTTGAGT	ATGTCTGTTA	TTAATTTTAC	AGGTAGTTCT	GGTCCATTGG	6240
TGAAAGTTTG	CGGCTTGCAG	AGCACAGAGG	CCGCAGAATG	TGCTCTAGAT	TCCGATGCTG	6300

ACTTGCTGGG TATTATATGT GTGCCCCAATA GAAAGAGAAC AATTGACCCG GTTATTGCAA	6360
GGAAAATTTT AAGTCTTGTA AAAGCATATA AAAATAGTTC AGGCACTCCG AAATACTTGG	6420
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TGCCAGTTAT TAAAAGACTC GTATTTCCAA AAGACTGCAA CATACTACTC AGTGCAGCTT	6600
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GCGTTATTGG TGTTGATGTA AGCGGAGGTG TGGAGACAAA TGGTGTAAAA GACTCTAACA	6840
AAATAGCAAA TTTCGTCAAA AATGCTAAGA AATAGGTTAT TACTGAGTAG TATTTATTTA	6900
AGTATTGTTT GTGCACTTGC CGATCTATGC GGTGTGAAAT ACCGCACAGA TGCGTAAGGA	6960
GAAAATACCG CATCAGGAAA TTGTAAACGT TAATATTTTG TTAAAATTCG CGTTAAATTT	7020
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TGGTACTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT	8040
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AGATGTTGAA TTAGATTAAA CTGAAGATAT ATAATTTATT GGAAAATACA TAGAGCTTTT	8160
TGTTGATGCG CTTAAGCGAT CAATTCAACA ACACCACCAG CAGCTCTGAT TTTTCTTCA	8220

GCCAACTTGG	AGACGAATCT	AGCTTTGACG	ATAACTGGAA	CATTTGGGAT	TCTACCCTTA	8280
CCCAAGATCT	TACCGTAACC	GGCTGCCAAA	GTGTCAATAA	CTGGAGCAGT	TTCCTTAGAA	8340
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CATACCTCTA	CCACCGGGGT	GCTTTCTGTG	CTTACCGATA	CGACCTTTAC	CGGCTGAGAC	8580
GTGACCTCTG	TGCTTTCTAG	TCTTAGTGAA	TCTGGAAGGC	ATTCTTGATT	AGTTGGATGA	8640
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AACGCCATCT	TAAATATACG	GGATACAGAT	GAAAGGTTTG	AACCTATCTG	GGAAAATACG	8760
CATTAAACAA	GCGAAAAACT	GCGAGGAAAA	TTGTTTGCGT	CTCTGCGGGC	TATTCACGCG	8820
CCAGAGGAAA	ATAGGAAAAA	TAACAGGGCA	TTAGAAAAAT	AATTTTGATT	TTGGTAATGT	8880
GTGGGTCCCT	GGTGTACAGA	TGTTACATTG	GTTACAGTAC	TCTTGTTTTT	GCTGTGTTTT	8940
TCGATGAATC	TCCAAAATGG	TTGTTAGCAC	ATGGAAGAGT	CACCGATGCT	AAGTTATCTC	9000
TATGTAAGCT	ACGTGGCGTG	ACTTTTGATG	AAGCCGCACA	AGAGATACAG	GATTGGCAAC	9060
TGCAAATAGA	ATCTGGGGAT	CTAGATATCC	TTTTGTTGTT	TCCGGGTGTA	CAATATGGAC	9120
TTCTCTTTTT	CTGGCAACCA	AACCCATACA	TCGGGATTCC	TATAATACCT	TCGTTGGTCT	9180
CCCTAACATG	TAGGTGGCGG	AGGGGAGATA	TACAATAGAA	CAGATACCAG	ACAAGACATA	9240
ATGGGCTAAA	CAAGACTACA	CCAATTACAC	TGCCTCATTG	ATGGTGGTAC	ATAACGAACT	9300
AATACTGTAG	CCCTAGACTT	GATAGCCATC	ATCATATCGA	AGTTTCACTA	CCCTTTTTCC	9360
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CTCTCTCCCC	CGTTGTTGTC	TCACCATATC	CGCAATGACA	AAAAAAATGA	TGGAAGACAC	9480
TAAAGGAAAA	AATTAACGAC	AAAGACAGCA	CCAACAGATG	TCGTTGTTCC	AGAGCTGATG	9540
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ATGAGCAACG	GTATACGGCC	TTCTTCCAG	TTACTTGAAT	TTGAAATAAA	AAAAGTTTGC	9660
CGCTTTGCTA	TCAAGTATAA	ATAGACCTGC	AATTATTAAT	CTTTTGTTTC	CTCGTCATTG	9720
TTCTCGTTCC	CTTTCTTCCT	TGTTTCTTTT	TCTGCACAAT	ATTTCAAGCT	ATACCAAGCA	9780
TACAATCAAC	TCCAAGCTTG	AAGCAAGCCT	CCTGAAAGAT	GAAGCTACTG	TCTTCTATCG	9840
AACAAGCATG	CGATATTTGC	CGACTTAAAA	AGCTCAAGTG	CTCCAAAGAA	AAACCGAAGT	9900
GCGCCAAGTG	TCTGAAGAAC	AACTGGGAGT	GTCGCTACTC	TCCCAAAACC	AAAAGGTCTC	9960
CGCTGACTAG	GGCACATCTG	ACAGAAGTGG	AATCAAGGCT	AGAAAGACTG	GAACAGCTAT	10020
TTCTACTGAT	TTTTCTTCGA	GAAGACCTTG	ACATGATTTT	GAAAATGGAT	TCTTTACAGG	10080
ATATAAAAGC	ATTGTTAACA	GGATTATTTG	TACAAGATAA	TGTGAATAAA	GATGCCGTCA	10140

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CAGATAGATT GGCTTCAGTG GAGACTGATA TGCCTCTAAC ATTGAGACAG CATAGAATAA	10200
GTGCGACATC ATCATCGGAA GAGAGTAGTA ACAAAGGTCA AAGACAGTTG ACTGTATCGC	10260
CGGAATTGCA ATACCCAGCT TTGACTCA	10288

## (2) INFORMATION FOR SEQ ID NO: 29:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7625 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: circular

## (ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "plasmid"

## (iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GCTTGCATGC AACTTCTTTT CTTTTTTTTT CTTTCTCTC TCCCCCGTTG TTGTCTCACC	60
ATATCCGCAA TGACAAAAAA AATGATGGAA GACACTAAAG GAAAAAATTA ACGACAAAGA	120
CAGCACCAAC AGATGTCGTT GTTCCAGAGC TGATGAGGGG TATCTTCGAA CACACGAAAC	180
TTTTTCCTTC CTTCAATCAC GCACACTACT CTCTAATGAG CAACGGTATA CGGCCTTCCT	240
TCCAGTTACT TGAATTTGAA ATAAAAAAG TTTGCCGCTT TGCTATCAAG TATAAATAGA	300
CCTGCAATTA TTAATCTTTT GTTTCCTCGT CATGTCTCTC GTTCCCTTTC TTCCTTGTTT	360
CTTTTTCTGC ACAATATTTT AAGCTATACC AAGCATACAA TCAACTCCAA GCTTTGCAAA	420
GATGGATAAA GCGGAATTAA TTCCCGAGCC TCCAAAAAAG AAGAGAAAGG TCGAATTGGG	480
TACCGCCGCC AATTTTAATC AAAGTGGGAA TATTGCTGAT AGCTCATTGT CCTTCACTTT	540
CACTAACAGT AGCAACGGTC CGAACCTCAT AACAACTCAA ACAAATCTCT AAGCGCTTTC	600
ACAACCAATT GCCTCCTCTA ACGTTCATGA TAACTTCATG AATAATGAAA TCACGGCTAG	660
TAAAATTGAT GATGGTAATA ATTCAAAACC ACTGTCACCT GGTGGACGG ACCAACTGC	720
GTATAACGCG TTTGGAATCA CTACAGGGAT GTTTAATACC ACTACAATGG ATGATGTATA	780
TAACTATCTA TTCGATGATG AAGATACCCC ACCAAACCCA AAAAAAGAGA TCGAATTCCC	840
GGGGATCCGC TCCTCACTCT CCAAGTTCAC CAAGAAGAAG AACAGAAGT ACGACGAAGC	900
ACATATGCCA TCAATTTCCG GATCTCAAGG AACTCTTGAC AACATTGATG TGATTGAGTT	960
GAAGCAAGAG CTCAAAGAAC GCGATAGTGC ACTTTACGAA GTCCGCCTTG ACAATCTGGA	1020
TCGTGCCCCG GAAGTTGATG TTCTGAGGGA GACAGTGAAC AAGTTGAAAA CCGAGAACAA	1080
GCAATTAAAG AAAGAAGTGG ACAAACTCAC CAACGGTCCA GCCACTCGTG CTTCTTCCCG	1140
CGCCTCAATT CCAGTTATCT ACGACGATGA GCATGTCTAT GATGCAGCGT GTAGCAGTAC	1200

ATCAGCTAGT	CAATCTTCGA	AACGATCCTC	TGGCTGCAAC	TCAATCAAGG	TTACTGTAAA	1260
CGTGGACATC	GCTGGAGAAA	TCAGTTCGAT	CGTTAACCCG	GACAAAGAGA	TAATCGTAGG	1320
ATATCTTGCC	ATGTCAACCA	GTCAGTCATG	CTGGAAAGAC	ATTGATGTTT	CTATTCTAGG	1380
ACTATTTGAA	GTCTACCTAT	CCAGAATTGA	TGTGGAGCAT	CAACTTGGAA	TCGATGCTCG	1440
TGATTCTATC	CTTGGCTATC	AAATTGGTGA	ACTTCGACGC	GTCATTGGAG	ACTCCACAAC	1500
CATGATAACC	AGCCATCCAA	CTGACATTCT	TACTTCCTCA	ACTACAATCC	GAATGTTTAT	1560
GCACGGTGCC	GCACAGAGTC	GCGTAGACAG	TCTGGTCCTT	GATATGCTTC	TTCCAAAGCA	1620
AATGATTCTC	CAACTCGTCA	AGTCAATTTT	GACAGAGAGA	CGTCTGGTGT	TAGCTGGAGC	1680
AACTGGAATT	GGAAAGAGCA	AACTGGCGAA	GACCTTGGCT	GCTTATGTAT	CTATTCGAAC	1740
AAATCAATCC	GAAGATAGTA	TTGTTAATAT	CAGCATTCCT	GAAAACAATA	AAGAAGAATT	1800
GCTTCAAGTG	GAACGACGCC	TGGAAAAGAT	CTATGAATCG	TAGATACTGA	AAAACCCCGC	1860
AAGTTCACTT	CAACTGTGCA	TCGTGCACCA	TCTCAATTTT	TTTCATTTAT	ACATCGTTTT	1920
GCCTTCTTTT	ATGTAACAT	ACTCCTCTAA	GTTTCAATCT	TGGCCATGTA	ACCTCTGATC	1980
TATAGAATTT	TTTAAATGAC	TAGAATTAAT	GCCCATCTTT	TTTTTGGACC	TAAATTCTTC	2040
ATGAAAATAT	ATTACGAGGG	CTTATTCAGA	AGCTTTGGAC	TTCTTCGCCA	GAGGTTTGGT	2100
CAAGTCTCCA	ATCAAGGTTG	TCGGCTTGTC	TACCTTGCCA	GAAATTTACG	AAAAGATGGA	2160
AAAGGGTCAA	ATCGTTGGTA	GATACGTTGT	TGACACTTCT	AAATAAGCGA	ATTTCTTATG	2220
ATTTATGATT	TTTATTATTA	AATAAGTTAT	AAAAAAAATA	AGTGTATACA	AATTTTAAAG	2280
TGACTCTTAG	GTTTTAAAC	GAAAATTCTT	GTTCTTGAGT	AACTCTTTCC	TGTAGGTCAG	2340
GTTGCTTTCT	CAGGTATAGC	ATGAGGTCGC	TCTTATTGAC	CACACCTCTA	CCGGCATGCC	2400
CGAAATTCCC	CTACCCTATG	AACATATTCC	ATTTTGTAAT	TTCGTGTCGT	TTCTATTATG	2460
AATTTCAATTT	ATAAAGTTTA	TGTACAAATA	TCATAAAAAA	AGAGAATCTT	TTTAAGCAAG	2520
GATTTTCTTA	ACTTCTTCGG	CGACAGCATC	ACCGACTTCG	GTGGTACTGT	TGGAACCACC	2580
TAAATCACCA	GTTCTGATAC	CTGCATCCAA	AACCTTTTTTA	ACTGCATCTT	CAATGGCCTT	2640
ACCTTCTTCA	GGCAAGTTCA	ATGACAATTT	CAACATCATT	GCAGCAGACA	AGATAGTGGC	2700
GATAGGGTCA	ACCTTATTCT	TTGGCAAATC	TGGAGCAGAA	CCGTGGCATG	GTTTCGTACAA	2760
ACCAAATGCG	GTGTTCTTGT	CTGGCAAAGA	GGCCAAGGAC	GCAGATGGCA	ACAAACCCAA	2820
GGAACCTGGG	ATAACGGAGG	CTTCATCGGA	GATGATATCA	CCAAACATGT	TGCTGGTGAT	2880
TATAATACCA	TTTAGGTGGG	TTGGGTTCTT	AACTAGGATC	ATGGCGGCAG	AATCAATCAA	2940
TTGATGTTGA	ACCTTCAATG	TAGGAAATTC	GTTCTTGATG	GTTTCCTCCA	CAGTTTTTCT	3000
CCATAATCTT	GAAGAGGCCA	AAACATTAGC	TTTATCCAAG	GACCAAATAG	GCAATGGTGG	3060
CTCATGTTGT	AGGGCCATGA	AAGCGGCCAT	TCTTGTGATT	CTTTGCACTT	CTGGAACGGT	3120



GTATTGTTCA CTATCCCAAG CGACACCATC ACCATCGTCT TCCTTTCTCT TACCAAAGTA	3180
AATACCTCCC ACTAATTCTC TGACAACAAC GAAGTCAGTA CCTTTAGCAA ATTGTGGCTT	3240
GATTGGAGAT AAGTCTAAAA GAGAGTCGGA TGCAAAGTTA CATGGTCTTA AGTTGGCGTA	3300
CAATTGAAGT TCTTTACGGA TTTTGTAGTA ACCTTGTTCA GGTCTAACAC TACCTGTACC	3360
CCATTTAGGA CCACCCACAG CACCTAACAA AACGGCATCA ACCTTCTTGG AGGCTTCCAG	3420
CGCCTCATCT GGAAGTGGGA CACCTGTAGC ATCGATAGCA GCACCACCAA TTAAATGATT	3480
TTCGAAATCG AACTTGACAT TGGAACGAAC ATCAGAAATA GCTTTAAGAA CCTTAATGGC	3540
TTCGGCTGTG ATTTCTTGAC CAACGTGGTC ACCTGGCAA ACGACGATCT TCTTAGGGGC	3600
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CTATATGAAA AGCCGGTTCC GGCCTCTCAC CTTTCCTTTT TCTCCCAATT TTTCAATTGA	3840
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CTAAGAGATT CGAACTCTTG CATCTTACGA TACCTGAGTA TTCCACAGT TGGGGATCTC	4020
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TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA TAAAGTGTA AGCCTGGGGT	4140
GCCTAATGAG TGAGGTAAC CACATTAATT GCGTTGCGCT CACTGCCCCG TTTCCAGTCG	4200
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CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT CGTTCGGCTG	4320
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AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT	4620
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CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT	5100
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GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA	5400
GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT CCGCCTCCAT CCAGTCTATT	5460
AATTGTTGCC GGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG CAACGTTGTT	5520
GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC	5580
GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCATGT TGTGCAAAAA AGCGGTTAGC	5640
TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG CAGTGTTATC ACTCATGGTT	5700
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GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GCGGACCGAG TTGCTCTTGC	5820
CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAGT GCTCATCATT	5880
GGAAAAAGTT CTTGGGGCG AAAACTCTCA AGGATCTTAC CGCTGTTGAG ATCCAGTTCCG	5940
ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTAATTTTAC CAGCGTTTCT	6000
GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAGG GAATAAGGGC GACACGGAAA	6060
TGTTGAATAC TCATACTCTT CCTTTTCAA TATTATTGAA GCATTTATCA GGGTTATTGT	6120
CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCGCGC	6180
ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA TTATTATCAT GACATTAACC	6240
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AGCAGACAAG CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG GGTGTCGGGG CTGGCTTAAC	6420
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GCACTATGCC GTTCTTCTCA TGTATATATA TATACAGGCA ACACGCAGAT ATAGGTGCGA	6540
CGTGAACAGT GAGCTGTATG TGCGCAGCTC GCGTTGCATT TTCGGAAGCG CTCGTTTTCG	6600
GAAACGCTTT GAAGTTCCTA TTCCGAAGTT CCTATTCTCT AGCTAGAAAG TATAGGAACT	6660
TCAGAGCGCT TTTGAAAACC AAAAGCGCTC TGAAGACGCA CTTTCAAAAA ACCAAAAACG	6720
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AAGTATCTCT TTGCTATATA TCTCTGTGCT ATATCCCTAT ATAACCTACC CATCCACCTT	6840
TCGCTCCTTG AACTTGCATC TAAACTCGAC CTCTACATTT TTTATGTTTA TCTCTAGTAT	6900
TACTCTTTAG ACAAAAAAAT TGTAAGTAAGA ACTATTCATA GAGTGAATCG AAAACAATAC	6960

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GAAAATGTAA ACATTTTCCTA TACGTAGTAT ATAGAGACAA AATAGAAGAA ACCGTTTCATA	7020
ATTTTCTGAC CAATGAAGAA TCATCAACGC TATCACTTTC TGTTCAACAA GTATGCGCAA	7080
TCCACATCGG TATAGAATAT AATCGGGGAT GCCTTTATCT TGAAAAAATG CACCCGCGAGC	7140
TTCGCTAGTA ATCAGTAAAC GCGGGAAGTG GAGTCAGGCT TTTTTTATGG AAGAGAAAAT	7200
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TCGTT	7625

## (2) INFORMATION FOR SEQ ID NO: 30:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9642 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "plasmid"

## (iii) HYPOTHETICAL: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

ATGACCATGA TTACGCCAAG CTTGTCTTCT TCTAAATTCC CATAAAATCC CGAAACTCCT	60
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AATTGAGTGG GATGAGGCTA TCTCTGCCTC TCTTCTGAAT CTCTGAACCA TCTTACATTA	180
CACTGTGGAT GACGAGCCCC ACAGGCTCCC TTGCATCAGA TACTGCCATT GGGGATGGCA	240
AAGAAGAGAG AAGGTATTGT GAGGATATAT TTTTCTAAGA AAAAACGTTT GAAGAAAAGA	300
AGATGAAGAA GATCTGCTTG ATTCATTGCA CAAGTTAGAA GTAACAGGGG TCTATATTTC	360
GAAGAACTTA AAGGGAATGC AACTGAACAT AAAATTAAAC AAAGGGATTG AATCCTGCAG	420
TGAGTATTTT CGGTTTTTCA CTGGTTCTCT GTAAAAAGAG TAATGCAAAG GGCAAGTTAA	480
CTTAGGTCGT AAATGTATTG AATTTGCTTA AAATCTGAAG ATCTAGTGGT GAACCGTGGA	540
AGATTATCAA GAGGAGGCTG AAGATCTGTT TAAGAACCAT TAATCAAACCT GGTATTCTAT	600
TTTCACTGGT TGTATGTAAA CATTCTATCT TATTCCTTTT ATCACTGTTC TGCACTTTCC	660

TATAAAAAAA	GTTGACCGAC	CGTACTCTCT	GAATTCATTT	TTCCCGATCT	TACCAACTCC	720
CGATCTATCT	CTATCCCTGG	TTTTTCTTC	GTGCTCCAAT	GGAATTCTTG	AGACTTCCAC	780
TATCTTCTCT	GGCACCTCC	ACTACGCGTA	GGCGTCTCTC	GCTTCGTGTA	TTCCCGGGAA	840
GCCGGTTCCC	GTCTCTCCCG	CCGCTGCCGC	TGCCGCACAC	AGCTTTACAC	CTCGTAGAAT	900
CCCCAAAGAG	GGGCGTGGCT	TGCGGGTGCC	AACATCCTCC	TGCCGAGGAA	GAAGCAGGCA	960
CTCATCACTC	GCATCATCAA	CCTCGGGATT	GGCCAAAGGA	CCCAAAGGTA	TGTTTCGAAT	1020
GATACTAACA	TAACATAGAA	CATTTTCAGG	AGGACCCTTG	GCTAGAACTA	GTGGATCCGA	1080
GCTCTCCCAT	ATGACGACGT	CAAATGTAGA	ATTGATACCA	ATCTACACGG	ATTGGGCCAA	1140
TCGGCACCTT	TCGAAGGGCA	GCTTATCAAA	GTCGATTAGG	GATATTTCCA	ATGATTTTCG	1200
CGACTATCGA	CTGGTTTCTC	AGCTTATTAA	TGTGATCGTT	CCGATCAACG	AATTCTCGCC	1260
TGCATTACAG	AAACGTTTGG	CAAAAATCAC	ATCGAACCTG	GATGGCCTCG	AAACGTGTCT	1320
CGACTACCTG	AAAAATCTGG	GTCTCGACTG	CTCGAAACTC	ACCAAACCG	ATATCGACAG	1380
CGGAAACTTG	GGTGCACTC	TCCAGCTGCT	CTTCCTGCTC	TCCACCTACA	AGCAGAAGCT	1440
TCGGCAACTG	AAAAAAGATC	AGAAGAAATT	GGAGCAACTA	CCCACATCCA	TTATGCCACC	1500
CGCGGTTTCT	AAATTACCCT	CGCCACGTGT	CGCCACGTCA	GCAACCGCTT	CAGCAACTAA	1560
CCCAAATTCC	AACTTTCCAC	AAATGTCAAC	ATCCAGGCTT	CAGACTCCAC	AGTCAAGAAT	1620
ATCGAAAATT	GATTCATCAA	AGATTGGTAT	CAAGCCAAAG	ACGTCTGGAC	TTAAACCACC	1680
CTCATCATCA	ACCACTTCAT	CAAATAATAC	AAATTCATTC	CGTCCGTCGA	GCCGTTTCGAG	1740
TGGCAATAAT	AATGTTGGCT	CGACGATATC	CACATCTGCG	AAGAGCTTAG	AATCATCATC	1800
AACGTACAGC	TCTATTTCTGA	ATCTAAACCG	ACCTACCTCC	CAACTCCAAA	AACCTTCTAG	1860
ACCACAAACC	CAGCTAGTTC	GTGTTGCTAC	AACTACAAAA	ATCGGAAGCT	CAAAGCTAGC	1920
CGCTCCGAAA	GCCGTGAGCA	CCCCAAAAC	TGCTTCTGTG	AAGACTATTG	GAGCAAAACA	1980
AGAGCCCGAT	AACAGCGGTG	GTGGTGGTGG	TGGAATGCTG	AAATTAAAGT	TATTCAGTAG	2040
CAAAAACCCA	TCTTCCTCAT	CGAATAGCCC	ACAACCTACG	AGAAAGGCGG	CGGCGGTGCC	2100
TCAACAACAA	ACTTTGTCTGA	AAATCGCTGC	CCCAGTGAAA	AGTGGCCTGA	AGCCGCCGAC	2160
CAGTAAGCTG	GGAAGTGCCA	CGTCTATGTC	GAAGCTTTGT	ACGCCAAAAG	TTTCTACCG	2220
TAAAACGGAC	GCCCCAATCA	TATCTCAACA	AGACTCGAAA	CGATGCTCAA	AGAGCAGTGA	2280
AGAAGAGTCC	GGATACGCTG	GATTCAACAG	CACGTCGCCA	ACGTCATCAT	CGACGGAAGG	2340
TTCCCTAAGC	ATGCATTCCA	CATCTTCCAA	GAGTTCAACG	TCAGACGAAA	AGTCTCCGTC	2400
ATCAGACGAT	CTTACTCTTA	ACGCCTCCAT	CGTGACAGCT	ATCAGACAGC	CGATAGCCGC	2460
AACACCGGTT	TCTCCAAATA	TTATCAACAA	GCCTGTTGAG	GAAAAACCAA	CACTGGCAGT	2520
GAAAGGAGTG	AAAAGCACAG	CGAAAAAAGA	TCCACCTCCA	GCTGTTCCGC	CACGTGACAC	2580

CCAGCCAACA	ATCGGAGTTG	TTAGTCCAAT	TATGGCACAT	AAGAAGTTGA	CAAATGACCC	2640
CGTGATATCT	GAAAAACCAG	AACCTGAAAA	GCTCCAATCA	ATGAGCATCG	ACACGACGGA	2700
CGTTCCACCG	CTTCCACCTC	TAAAATCAGT	TGTTCCACTT	AAAATGACTT	CAATCCGACA	2760
ACCACCAACG	TACGATGTTC	TTCTAAAACA	AGGAAAAATC	ACATCGCCTG	TCAAGTCGTT	2820
TGGATATGAG	CAGTCGTCCG	CGTCTGAAGA	CTCCATTGTG	GCTCATGCGT	CGGCTCAGGT	2880
GACTCCGCCG	ACAAAAACTT	CTGGTAATCA	TTCGCTGGAG	AGAAGGATGG	GAAAGAATAA	2940
GACATCAGAA	TCCAGCGGCT	ACACCTCTGA	CGCCGGTGTT	GCGATGTGCG	CCAAAATGAG	3000
GGAGAAGCTG	AAAGAATACG	ATGACATGAC	TCGTCGAGCA	CAGAACGGCT	ATCCTGACAA	3060
CTTCGAAGAC	AGTTCCTCCT	TGTCGTCTGG	AATATCCGAT	AACAACGAGC	TCGACGACAT	3120
ATCCACGGAC	GATTTGTCCG	GAGTAGACAT	GGCAACAGTC	GCCTCCAAAC	ATAGCGACTA	3180
TTCCCACTTT	GTTCCGCATC	CCACGTCTTC	TTCTCAAAG	CCCCGAGTCC	CCAGTCGGTC	3240
CTCCACATCA	GTCGATTCTC	GATCTCGAGC	AGAACAGGAG	AATGTGTACA	AACTTCTGTC	3300
CCAGTGCCGA	ACGAGCCAAC	GTGGCGCCGC	TGCCACCTCA	ACCTTCGGAC	AACATTCGCT	3360
AAGATCCCCG	GGATACTCAT	CCTATTCTCC	ACACTTATCA	GTGTCAGCTG	ATAAGGACAC	3420
AATGTCTATG	CACTCACAGA	CTAGTCGACG	ACCTTCTTCA	CAAAAACCAA	GCTATTCAGG	3480
CCAATTTTCAT	TCACTTGATC	GTAAATGCCA	CCTTCAAGAG	TTACATCCA	CCGAGCACAG	3540
AATGGCGGCT	CTCTTGAGCC	CGAGACGGGT	GCCGAACTCG	ATGTCGAAAT	ATGATTCTTC	3600
AGGATCCTAC	TCGGCGCGTT	CCCGAGGTGG	AAGCTCTACT	GGTATCTATG	GAGAGACGTT	3660
CCAACTGCAC	AGACTATCCG	ATGAAAAATC	CCCCGCACAT	TCTGCCAAAA	GTGAGATGGG	3720
ATCCCAACTA	TCACTGGCTA	GCACGACAGC	ATATGGATCT	CTCAATGAGA	AGTACGAACA	3780
TGCTATTTCG	GACATGGCAC	GTGACTTGGA	GTGTTACAAG	AACACTGTCG	ACTCACTAAC	3840
CAAGAAACAG	GAGAACTATG	GAGCATTGTT	TGATCTTTTT	GAGCAAAAGC	TTAGAAAAC	3900
CACTCAACAC	ATTGATCGAT	CCAACCTGAA	GCCTGAAGAG	GCAATACGAT	TCAGGCAGGA	3960
CATTGCTCAT	TTGAGGGATA	TTAGCAATCA	TCTTGCATCC	AACTCAGCTC	ATGCTAACGA	4020
AGGCGCTGGT	GAGCTTCTTC	GTCAACCATC	TCTGGAATCA	GTTGCATCCC	ATCGATCATC	4080
GATGTCATCG	TCGTCGAAAA	GCAGCAAGCA	GGAGAAGATC	AGCTTGAGCT	CGTTTGGCAA	4140
GAACAAGAAG	AGCTGGATCC	GCTCCTCACT	CTCCAAGTTC	ACCAAGAAGA	AGAACAAGAA	4200
CTACGACGAA	GCACATATGC	CATCAATTTT	CGGATCTCAA	GGAACCTCTG	ACAACATTGA	4260
TGTGATTGAG	TTGAAGCAAG	AGCTCAAAGA	ACGCGATAGT	GCACTTTACG	AAGTCCGCCT	4320
TGACAATCTG	GATCGTGCCC	GCGAAGTTGA	TGTTCTGAGG	GAGACAGTGA	ACAAGTTGAA	4380
AACCGAGAAC	AAGCAATTAA	AGAAAGAAGT	GGACAAACTC	ACCAACGGTC	CAGCCACTCG	4440
TGCTTCTTCC	CGCGCCTCAA	TTCCAGTTAT	CTACGACGAT	GAGCATGTCT	ATGATGCAGC	4500

GTGTAGCAGT	ACATCAGCTA	GTCAATCTTC	GAAACGATCC	TCTGGCTGCA	ACTCAATCAA	4560
GGTTACTGTA	AACGTGGACA	TCGCTGGAGA	AATCAGTTCC	ATCGTTAACC	CGGACAAAGA	4620
GATAATCGTA	GGATATCTTG	CCATGTCAAC	CAGTCAGTCA	TGCTGGAAAG	ACATTGATGT	4680
TTCTATTCTA	GGACTATTTG	AAGTCTACCT	ATCCAGAATT	GATGTGGAGC	ATCAACTTGG	4740
AATCGATGCT	CGTGATTCTA	TCCTTGGCTA	TCAAATTGGT	GAACCTCGAC	GCGTCATTGG	4800
AGACTCCACA	ACCATGATAA	CCAGCCATCC	AACTGACATT	CTTACTTCCT	CAACTACAAT	4860
CCGAATGTTC	ATGCACGGTG	CCGCACAGAG	TCGCGTAGAC	AGTCTGGTCC	TTGATATGCT	4920
TCTTCCAAAG	CAAATGATTC	TCCAACTCGT	CAAGTCAATT	TTGACAGAGA	GACGTCTGGT	4980
GTTAGCTGGA	GCAACTGGAA	TTGGAAAGAG	CAAACTGGCG	AAGACCCTGG	CTGCTTATGT	5040
ATCTATTTCGA	ACAAATCAAT	CCGAAGATAG	TATTGTTAAT	ATCAGCATTG	CTGAAAACAA	5100
TAAAGAAGAA	TTGCTTCAAG	TGGAACGACG	CCTGGAAAAG	ATCTTGAGAA	GCAAAGAATC	5160
ATGCATCGTA	ATTCTAGATA	ATATCCCAAA	GAATCGAATT	GCATTTGTTG	TATCCGTTTT	5220
TGCAAATGTC	CCACTTCAAA	ACAACGAAGG	TCCATTTGTA	GTATGCACAG	TCAACCGATA	5280
TCAAATCCCT	GAGCTTCAAA	TTCACCACAA	TTTCAAAATG	TCAGTAATGT	CGAATCGTCT	5340
CGAAGGATTC	ATCCTACGTT	ACCTCCGACG	ACGGGCGGTA	GAGGATGAGT	ATCGTCTAAC	5400
TGTACAGATG	CCATCAGAGC	TCTTCAAAAT	CATTGACTTC	TTCCCAATAG	CTCTTCAGGC	5460
CGTCAATAAT	TTTATTGAGA	AAACGAATTC	TGTTGATGTG	ACAGTTGGTC	CAAGAGCATG	5520
CTTGAAGTGT	CCTCTAACTG	TCGATGGATC	CCGTGAATGG	TTCATTCGAT	TGTGGAATGA	5580
GAACCTCATT	CCATATTTGG	AACGTGTTGC	TAGAGATGGC	AAAAAAACCT	TCGGTCGCTG	5640
CACTTCCTTC	GAGGATCCCA	CCGACATCGT	CTCTAAAAAA	TGGCCGTGGT	TCGATGGTGA	5700
AAACCCGGAG	AATGTGCTCA	AACGTCTTCA	ACTCCAAGAC	CTCGTCCCGT	CACCTGCCAA	5760
CTCATCCCGA	CAACACTTCA	ATCCCCCTGA	GTCGTTGATC	CAATTGCATG	CTACCAAGCA	5820
TCAGACCATC	GACAACATTT	GAACAGAAGA	CTCTAATCTT	CTCTCGCCTC	TCCCCCGCTT	5880
TCCTTATCTT	CGTACCGGTA	CCATGGTATT	GATATCTGAG	CTCCGCATCG	GCCGCTGTCA	5940
TCAGATCGCC	ATCTCGCGCC	CGTGCCCTCG	ACTTCTAAGT	CCAATTACTC	TTCAACATCC	6000
CTACATGCTC	TTTCTCCCTG	TGCTCCACCC	CCCTATTTTT	GTTATTATCA	AAAAAACTTC	6060
TTCTTAATTT	CTTTGTTTTT	TAGCTTCTTT	TAAGTCACCT	CTAACAATGA	AATTGTGTAG	6120
ATTCAAAAAAT	AGAATTAATT	CGTAATAAAA	AGTCGAAAAA	AATTGTGCTC	CCTCCCCCCA	6180
TTAATAATAA	TTCTATCCCA	AAATCTACAC	AATGTTCTGT	GTACACTTCT	TATGTTTTTT	6240
TTACTTCTGA	TAAATTTTTT	TTGAAACATC	ATAGAAAAAA	CCGCACACAA	AATACCTTAT	6300
CATATGTTAC	GTTTCAGTTT	ATGACCGCAA	TTTTTATTTT	TTGCGACGTC	TGGGCCTCTC	6360
ATGACGTCAA	ATCATGCTCA	TCGTGAAAAA	GTTTTGGAGT	ATTTTTGGAA	TTTTTCAATC	6420

AAGTGAAAGT	TTATGAAATT	AATTTTCCTG	CTTTTGCTTT	TTGGGGGTTT	CCCCTATTGT	6480
TTGTCAAGAG	TTTCGAGGAC	GGCGTTTTTC	TTGCTAAAAT	CACAAGTATT	GATGAGCACG	6540
ATGCAAGAAA	GATCGGAAGA	AGGTTTGGGT	TTGAGGCTCA	GTGGAAGGTG	AGTAGAAGTT	6600
GATAATTTGA	AAGTGGAGTA	GTGTCTATGG	GGTTTTTGCC	TTAAATGACA	GAATACATTC	6660
CCAATATACC	AAACATAACT	GTTTAAAATT	AAACATTTTT	CTAAATTTTA	TATGATTTCT	6720
TTTAAATTTG	CAAAAATTAC	TTAAATTTGA	ATTCCCGCGC	AAATGAGTGA	CTTCATTTTC	6780
TGCATTATTG	TGTTTTCCGG	CTATATTAAT	AGGTATTTGT	TTGTGTTTTT	CTTTATTTTA	6840
TGATTGGAAC	TCCAATTTGT	AAATTTTCGA	ACATATTTCC	CTAAAGAAAA	AATATGATTA	6900
ATCTGGAAAA	ATTGGAAAAT	TATTTTTCAA	ATAAAAAACA	AAGAAAAAAA	TGAAGAAAAA	6960
CCTATTAGTT	TGGCCATAAA	ACGCAAAAAT	GTCGAAAATG	ACGTCACTCA	TCTGCGCGGG	7020
AAATCAAGAA	TAATTCGGCC	TTTTTTATTT	TTTTGGAAAA	TCGTAAAACA	TTTAGAAAAA	7080
TTTTTTAATA	GTTATAGTGG	GACTGTATTC	TGTCATTTAG	GGCAAAAGCC	AGAGACGCTA	7140
CTCCACCGTT	GGGGGATCCA	CTAGTCGGCC	GTACGGGCCC	TTTCGTCTCG	CGCGTTTCGG	7200
TGATGACGGT	GAAAACCTCT	GACACATGCA	GCTCCCGGAG	ACGGTCACAG	CTTGTCTGTA	7260
AGCGGATGCC	GGGAGCAGAC	AAGCCCGTCA	GGGCGCGTCA	GCGGGTGTTG	GCGGGTGTCG	7320
GGGCTGGCTT	AACTATGCGG	CATCAGAGCA	GATTGTACTG	AGAGTGCACC	ATATGCGGTG	7380
TGAAATACCG	CACAGATGCG	TAAGGAGAAA	ATACCGCATC	AGGCGGCCTT	AAGGGCCTCG	7440
TGATACGCCT	ATTTTTATAG	GTTAATGTCA	TGATAATAAT	GGTTTCTTAG	ACGTCAGGTG	7500
GCACTTTTCG	GGGAAATGTG	CGCGGAACCC	CTATTTGTTT	ATTTTTCTAA	ATACATTCAA	7560
ATATGTATCC	GCTCATGAGA	CAATAACCCT	GATAAATGCT	TCAATAATAT	TGAAAAAGGA	7620
AGAGTATGAG	TATTCAACAT	TTCCGTGTCT	CCCTTATTCC	CTTTTTTGCG	GCATTTTGCC	7680
TTCCTGTTTT	TGCTCACCCA	GAAACGCTGG	TGAAAGTAAA	AGATGCTGAA	GATCAGTTGG	7740
GTGCACGAGT	GGGTTACATC	GAAGTGGATC	TCAACAGCGG	TAAGATCCTT	GAGAGTTTTC	7800
GCCCCGAAGA	ACGTTTTCCA	ATGATGAGCA	CTTTTAAAGT	TCTGCTATGT	GGCGCGGTAT	7860
TATCCCGTAT	TGACGCCGGG	CAAGAGCAAC	TCGGTCGCCG	CATACACTAT	TCTCAGAAATG	7920
ACTTG GTTGA	GTA CTACCA	GTCACAGAAA	AGCATCTTAC	GGATGGCATG	ACAGTAAGAG	7980
AATTATGCAG	TGCTGCCATA	ACCATGAGTG	ATAACACTGC	GGCCAACTTA	CTTCTGACAA	8040
CGATCGGAGG	ACCGAAGGAG	CTAACCGCTT	TTTTGCACAA	CATGGGGGAT	CATGTAACCTC	8100
GCCTTGATCG	TTGGGAACCG	GAGCTGAATG	AAGCCATACC	AAACGACGAG	CGTGACACCA	8160
CGATGCCTGT	AGCAATGGCA	ACAACGTTGC	GCAAACTATT	AACTGGCGAA	CTACTTACTC	8220
TAGCTTCCCG	GCAACAATTA	ATAGACTGGA	TGGAGGCGGA	TAAAGTTGCA	GGACCACTTC	8280
TGCGCTCGGC	CCTTCCGGCT	GGCTGGTTTA	TTGCTGATAA	ATCTGGAGCC	GGTGAGCGTG	8340

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GGTCTCGCGG TATCATTGCA GCACTGGGGC CAGATGGTAA GCCCTCCCGT ATCGTAGTTA      8400
TCTACACGAC GGGGAGTCAG GCAACTATGG ATGAACGAAA TAGACAGATC GCTGAGATAG      8460
GTGCCTCACT GATTAAGCAT TGGTAACTGT CAGACCAAGT TTACTCATAT ATACTTTAGA      8520
TTGATTTAAA ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT TTTGATAATC      8580
TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA      8640
AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA      8700
AAAAACCACC GCTACCAGCG GTGGTTTGTG TGCCGGATCA AGAGCTACCA ACTCTTTTTT      8760
CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCTTCTA GTGTAGCCGT      8820
AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC      8880
TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC      8940
GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTTCGTGC ACACAGCCCCA      9000
GCTTGGAGCG AACGACCTAC ACCGAAGTGA GATACCTACA GCGTGAGCAT TGAGAAAGCG      9060
CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG      9120
GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTGCGGT      9180
TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT      9240
GGAAAAACGC CAGCAACGCG GCCTTTTTTAC GGTTCCTGGC CTTTGTGCTG CCTTTTGCTC      9300
ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT      9360
GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG      9420
CGGAAGAGCG CCAATACGC AAACCGCCTC TCCCCGCGCG TTGGCCGATT CATTAATGCA      9480
GCTGGCACGA CAGGTTTCCC GACTGGAAAG CGGGCAGTGA GCGCAACGCA ATTAATGTGA      9540
GTTAGCTCAC TCATTAGGCA CCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT      9600
GTGGAATTGT GAGCGGATAA CAATTCACA CAGGAAACAG CT                          9642

```

## (2) INFORMATION FOR SEQ ID NO: 31:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 110 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

```

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp Ala
1           5           10           15
Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg Asp Ile
20           25           30

```



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Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln Leu Ile Asn Val  
 35 40 45

Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr Lys Arg Leu Ala  
 50 55 60

Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr Cys Leu Asp Tyr Leu  
 65 70 75 80

Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu Thr Lys Thr Asp Ile Asp  
 85 90 95

Ser Gly Asn Leu Gly Ala Val Leu Gln Leu Leu Phe Leu Leu  
 100 105 110

## (2) INFORMATION FOR SEQ ID NO: 32:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Lys Gln Lys Leu Arg Gln Leu Lys Lys Asp Gln Lys Lys Leu Glu Gln  
 1 5 10 15

Leu Pro Thr Ser  
 20

## (2) INFORMATION FOR SEQ ID NO: 33:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 9 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Asp Pro Pro Pro Ala Val Pro Pro Arg  
 1 5

## (2) INFORMATION FOR SEQ ID NO: 34:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 9 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Asp Val Pro Pro Leu Pro Pro Leu Lys  
 1 5

(2) INFORMATION FOR SEQ ID NO: 35:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 5 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Lys Lys Lys Asn Lys  
 1 5

(2) INFORMATION FOR SEQ ID NO: 36:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 20 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Lys Thr Glu Asn Lys Gln Leu Lys Lys Glu Val Asp Lys Leu Thr Asn  
 1 5 10 15

Gly Pro Ala Thr  
 20

(2) INFORMATION FOR SEQ ID NO: 37:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 8 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

Gly Ala Thr Gly Ile Gly Lys Ser  
 1 5

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## (2) INFORMATION FOR SEQ ID NO: 38:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 58 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

```

Met Ser Glu Glu Pro Thr Pro Val Ser Gly Asn Asp Lys Gln Leu Leu
 1             5             10             15
Asn Lys Ala Trp Glu Ile Thr Gln Lys Lys Thr Phe Thr Ala Trp Cys
          20             25             30
Asn Ser His Leu Arg Lys Leu Gly Ser Ser Ile Glu Gln Ile Asp Thr
          35             40             45
Asp Phe Thr Asp Gly Ile Lys Leu Ala Gln
          50             55

```

## (2) INFORMATION FOR SEQ ID NO: 39:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 44 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

```

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp Ala
 1             5             10             15
Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg Asp Ile
          20             25             30
Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln
          35             40

```

## (2) INFORMATION FOR SEQ ID NO: 40:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

```

Phe Glu Arg Ser Arg Ile Lys Ala Leu Ala Asp Glu Arg Glu Val Val
1           5           10           15
Gln Lys Lys Thr Phe Thr Lys Trp Val Asn Ser His Leu Ala Arg Val
          20           25           30
Ser Cys Arg Ile Thr Asp Leu Tyr Lys Asp Leu Arg Asp Gly Arg Met
          35           40           45
Leu Ile Lys
          50

```

(2) INFORMATION FOR SEQ ID NO: 41:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 59 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

```

Leu Leu Glu Val Ile Ser Asn Asp Pro Val Phe Lys Val Asn Lys Thr
1           5           10           15
Pro Lys Leu Arg Arg Ile His Asn Ile Gln Asn Val Gly Leu Cys Leu
          20           25           30
Lys His Ile Glu Ser His Gly Val Lys Leu Val Gly Ile Gly Ala Glu
          35           40           45
Glu Leu Val Asp Lys Asn Leu Lys Met Thr Leu
          50           55

```

(2) INFORMATION FOR SEQ ID NO: 42:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 60 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

```

Leu Ile Asn Val Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr
1           5           10           15
Lys Arg Leu Ala Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr Cys
          20           25           30
Leu Asp Tyr Leu Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu Thr Lys
          35           40           45
Thr Asp Ile Asp Ser Gly Asn Leu Gly Ala Val Leu
          50           55           60

```

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## (2) INFORMATION FOR SEQ ID NO: 43:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

```

Leu Leu Glu Val Leu Ser Gly Glu Met Leu Pro Lys Pro Thr Lys Gly
1           5           10           15
Lys Met Arg Ile His Cys Leu Glu Asn Val Asp Lys Ala Leu Gln Phe
          20           25           30
Leu Lys Glu Gln Arg Val His Leu Glu Asn Met Gly Ser His Asp Ile
          35           40           45
Val Asp Gly Asn His Arg Leu Val Leu
          50           55

```

## (2) INFORMATION FOR SEQ ID NO: 44:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

```

Gly Met Ile Trp Thr Ile Ile Leu Arg Phe Ala Ile Gln Asp Ile Ser
1           5           10           15
Ile Glu Glu Leu Ser Ala Lys Glu Ala Leu Leu Leu Trp Cys Gln Arg
          20           25           30
Lys Thr Glu Gly Tyr Asp Arg Val Lys Val
          35           40

```

## (2) INFORMATION FOR SEQ ID NO: 45:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 46 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

160

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

Gln Leu Leu Phe Leu Leu Ser Thr Tyr Lys Gln Lys Leu Arg Gln Leu  
 1 5 10 15  
 Lys Lys Asp Gln Lys Lys Leu Glu Gln Leu Pro Thr Ser Ile Met Pro  
 20 25 30  
 Pro Ala Val Ser Lys Leu Pro Ser Pro Arg Val Ala Thr Ser  
 35 40 45

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Gly Leu Ile Trp Thr Ile Ile Leu Arg Phe Gln Ile Gln Asp Ile Val  
 1 5 10 15  
 Val Gln Thr Gln Glu Gly Arg Glu Thr Arg Ser Ala Lys Asp Ala Leu  
 20 25 30  
 Leu Gln Phe Leu Lys Glu Gln Arg Val His Leu Glu Asn Met Gly Ser  
 35 40 45

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 100 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cosmid DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

GATCAGAAGA AATTGGAGCA ACTACCCACA TCCATTATGC CACCCGCGGT TTCTAAGTGA 60  
 GTTTAATTTT GAGTTTACGA CTACAAAAAT GTGTTCTTTA 100

161

## (2) INFORMATION FOR SEQ ID NO: 48:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 91 base pairs  
    (B) TYPE: nucleic acid  
    (C) STRANDEDNESS: single  
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cosmid DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

CCGCCTTCTG ACTTCGTGAC GACAGTCTCG ACACGTGGGG TTGCAGGTAG GAGTGGATGA	60
GTCGAAACTG ATAAGATAGT CATTTGAGAT C	91

CLAIMS:

1. A cDNA encoding an UNC-53 protein of C. elegans or a functional equivalent derivative fragment or bioprecursor of said protein, which cDNA comprises at least from nucleotide position 431 to nucleotide position 4647 of the sequence shown in Figure 1.
2. A cDNA as claimed in claim 1 comprising at least from nucleotide position 431 to the 3' end of the sequence shown in Figure 1.
3. A cDNA as claimed in Claim 1 comprising at least from nucleotide position 64 to nucleotide position 4647 of the sequence as shown in Figure 1.
4. A cDNA as claimed in claim 3 comprising at least from nucleotide position 64 to the 3' end of the sequence shown in Figure 1.
5. A cDNA as claimed in Claims 1 to 4 comprising the nucleotide sequence shown in Figure 1.
6. A cDNA encoding an UNC-53 protein of C. elegans or a functional equivalent, derivative, fragment or bioprecursor of said protein, which cDNA comprises at least from nucleotide position 431 to nucleotide position 4812 of the 7A variant of the sequence shown in Figure 2.
7. A cDNA as claimed in claim 6 comprising at least



from nucleotide position 431 to the 3' end of the 7A variant of the sequences shown in figure 2.

5        8.    A cDNA as claimed in Claim 6 comprising at least  
from nucleotide position 64 to nucleotide position  
4812 of the sequence shown in Figure 2.

10       9.    A cDNA as claimed in claim 8 comprising at least  
from nucleotide position 64 to the 3' end of the 7A  
variant of the sequence shown in figure 2.

15       10.   A cDNA as claimed in any of claims 6 to 9  
comprising the nucleotide sequence of the 7A variant  
of the sequence shown in Figure 2.

11.    A DNA expression vector which comprises a cDNA  
as claimed in any one of Claims 1 to 10.

20       12.   A host cell transformed or transfected with the  
vector of Claim 11.

13.    A host cell as claimed in Claim 12 which is a  
bacterial, an animal, a plant or an insect cell.

25       14.   A transgenic cell comprising a transgene capable  
of expressing UNC-53 protein of C. elegans or a  
functional equivalent, derivative, fragment or  
bioprecursor of said protein.

30       15.   A transgenic cell as claimed in Claim 14 which

cell is a C. elegans cell, an N4 neuroblastoma cell or an MCF-7 breast carcinoma cell.

5 16. A transgenic organism comprising a transgene capable of expressing UNC-53 protein of C. elegans or a functional equivalent, derivative, fragment or bioprecursor of said protein.

10 17. A transgenic organism as claimed in Claim 16 wherein said organism is C. elegans.

15 18. A transgenic organism as claimed in Claim 16 wherein said organism is an insect, a non-human mammal or a plant.

19. A mutant of C. elegans which comprises an induced mutation in the wild-type unc-53 gene, which mutation affects the regulation of cell motility or the shape or direction of cell migration.

20

20. An UNC-53 protein encoded by the cDNA of Claim 1 and which protein has the amino acid sequence shown in Figure 4 from amino acid position 135 to amino acid position 1528.

25

21. An UNC-53 protein encoded by the cDNA sequence of any of Claims 2 to 5 and which protein has the amino acid sequence shown in Figure 4.

30 22. An UNC-53 protein encoded by the cDNA sequence of Claim 6 and which protein has the amino acid

sequence shown in Figure 6 from amino acid position 135 to amino acid position 1583.

23. An UNC-53 protein encoded by the cDNA sequence according to any of Claims 7 to 10 and which protein has the amino acid sequence shown in Figure 6.

24. An UNC-53 protein of C. elegans, or a functional equivalent, derivative, fragment or bioprecursor of said protein, for use as a medicament to promote neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

25. An UNC-53 protein as claimed in any one of Claims 20 to 23 for use as a medicament to promote neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

26. Use of an UNC-53 protein of C. elegans, or a functional equivalent, derivative, fragment or bioprecursor of said protein in the manufacture of a medicament for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

27. Use of an UNC-53 protein as claimed in any one of Claims 20 to 23 in the manufacture of a medicament for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative or acute traumatic injuries.

28. A pharmaceutical composition comprising an UNC-53 protein of C. elegans, a functional equivalent, derivative, bioprecursor or fragment of said protein and an acceptable carrier, diluent or excipient therefor.

29. A pharmaceutical composition as claimed in Claim 28 which comprises an UNC-53 protein as claimed in any one of Claims 20 to 23.

30. A nucleic acid sequence encoding an UNC-53 protein of C. elegans or a functional fragment, equivalent, derivative or bioprecursor of said protein, for use as a medicament to promote neuronal regeneration, vascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

31. A nucleic acid sequence for use as claimed in Claim 27 wherein said sequence is a cDNA sequence as claimed in any one of Claims 1 to 10 or a functional fragment of said nucleic acid sequence.

32. Use of a nucleic acid sequence encoding an UNC-53 protein of C. elegans or a functional equivalent fragment, derivative or bioprecursor of said protein, in the manufacture of a medicament to promote neuronal regeneration, vascularization or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

33. Use of a nucleic acid sequence as claimed in Claim 32 wherein said sequence is a cDNA sequence as

claimed in any one of Claims 1 to 10 or a functional fragment of said nucleic acid sequence.

34. A pharmaceutical composition comprising a  
5 nucleic sequence acid encoding an UNC-53 protein of C. elegans or a functional equivalent, derivative fragment or bioprecursor of said protein and an acceptable carrier, diluent, or excipient therefor.

10 35. A pharmaceutical composition as claimed in Claim 34 wherein said nucleic acid sequence is a cDNA sequence as claimed in any one of Claims 1 to 10.

15 36. A method of determining whether a compound is an inhibitor or an enhancer of the regulation of cell shape or motility or the direction of cell migration, which method comprises contacting said compound with a transgenic cell as claimed in Claims 14 or 15 and screening for a phenotypic change in said cell.

20 37. A method as claimed in Claim 36 wherein said compound is an inhibitor or an enhancer of a protein of the signal transduction pathway of said transgenic cell of which pathway UNC-53 protein or a functional  
25 equivalent, fragment or bioprecursor thereof is a component or said compound is an inhibitor or an enhancer of a parallel or redundant signal transduction pathway in said cell.

30 38. A method as claimed in Claim 36 or 37 wherein said protein is UNC-53 protein or a functional equivalent, fragment, derivative or bioprecursor thereof.

39. A method as claimed in any of Claims 36 to 38 wherein said phenotypic change to be screened is a change in cell shape or a change in cell motility.

5 40. A method as claimed in any of claims 36 to 38 wherein said phenotypic change to be screened is a change in filipodia outgrowth, ruffling behaviour, cell adhesion or the length of neurite growth.

10 41. A method as claimed in any of Claims 36 to 40 wherein said transgenic cell is an N4 neuroblastoma cell and the phenotypic change to be screened is the length of neurite growth.

15 42. A method as claimed in any of Claims 36 to 40 wherein said transgenic cell is an MCF-7 breast carcinoma cell and the phenotypic change to be screened is the extent of phagokinesis.

20 43. A method of determining whether a compound is an inhibitor or an enhancer of the regulation of cell shape or motility or of the direction of cell migration which method comprises administering said compound to a transgenic organism as claimed in any  
25 one of Claims 16 to 20, or a mutant organism as claimed in Claim 19, and screening for a phenotypic change in said organism.

30 44. A method as claimed in Claim 43 wherein said compound is an inhibitor or enhancer of a protein of the signal transduction pathway of said transgenic or mutant organisms, of which pathway UNC-53 protein or a functional equivalent, derivative or bioprecursor

thereof is a component or said compound is an inhibitor or an enhancer of a parallel or redundant signal transduction pathway in said cell.

5     45. A method as claimed in Claim 44 wherein said protein of the signal transduction pathway is UNC-53 protein itself or a functional equivalent, fragment, derivative or bioprecursor of said protein.

10     46. A compound which is identifiable by the method according to any one of Claims 36 to 45 as an enhancer of the regulation of cell shape or motility or the direction of cell migration for use as a medicament for promoting neuronal regeneration, revascularisation  
15     or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

20     47. Use of a compound identifiable by the method of any one of Claims 36 to 45 as an enhancer of the regulation of cell shape or motility or the direction of cell migration in C. elegans in the manufacture of a medicament for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute  
25     traumatic injuries.

30     48. A pharmaceutical composition comprising the compound as claimed in Claim 46 and an acceptable carrier, diluent or excipient therefor.

49. A compound which is identifiable by the method according to any one of Claims 36 to 45 as an inhibitor of the regulation of cell motility or shape

or the direction of cell migration of C. elegans for use as a medicament for alleviating the spread of disease inducing cells or metastasis.

- 5      50. Use of a compound identifiable by the method according to any one of Claims 36 to 45 in the manufacture of a medicament for alleviating the spread of disease inducing cells or metastasis.
- 10     51. A pharmaceutical composition comprising the compound as claimed in Claim 49 and an acceptable carrier diluent or excipient therefor.
- 15     52. A transgenic cell which has been constructed to comprise a promoter sequence of an unc-53 gene of C. elegans fused to a nucleic acid sequence encoding a reporter molecule.
- 20     53. A transgenic cell as claimed in Claim 52 wherein said reporter molecule is green fluorescent protein (GFP).
- 25     54. A method of determining whether a compound is an inhibitor or an enhancer of transcription of an unc-53 gene in C. elegans or a functional fragment of said gene, which method comprises the steps of (a) contacting said compound with a transgenic cell according to Claim 52 and (b) monitoring of said reporter molecule and comparing the results obtained
- 30     from said monitoring step with a control comprising a transgenic cell as claimed in Claim 48, which cell has not been contacted with said compound.



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55. A method as claimed in Claim 54 wherein said reporter molecule detected is mRNA.

56. A method as claimed in Claim 54 wherein said  
5 reporter molecule detected is green fluorescent protein (GFP).

57. A compound which is identifiable by the method according to any one of Claims 54 to 56, as an  
10 enhancer of transcription of an unc-53 gene of C. elegans or a functional fragment of said gene for use in promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute  
15 traumatic injuries.

58. Use of a compound which is identifiable by the method of any one of Claims 54 to 56 as an enhancer of transcription of an unc-53 gene of C. elegans or a  
20 functional fragment of said gene in the manufacture of a medicament for promoting neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neuro-degenerative diseases or acute traumatic injuries.

25

59. A pharmaceutical composition which comprises the compound of Claim 57 and an acceptable carrier, diluent or excipient therefor.

30 60. A compound which is identifiable by the method of any one of Claims 54 to 56 as an inhibitor of transcription of an unc-53 gene of C. elegans or a functional fragment of said gene for use in

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alleviating the spread of disease inducing cells or metastasis.

5 61. Use of a compound which is identifiable by the method of any one of Claims 54 to 56 as an inhibitor of transcription of an unc-53 gene of C. elegans or a functional fragment of said gene in the manufacture of a medicament for alleviating spread of disease inducing cells or metastasis.

10

62. A pharmaceutical composition which comprises the compound of Claim 60 and an acceptable carrier, diluent or excipient therefor.

15

63. A kit for determining whether a compound is an enhancer or an inhibitor of the regulation of cell motility or shape or the direction of cell migration which kit comprises at least a plurality of transgenic cells as claimed in any one of Claims 14 or 15 and a plurality of wild-type cells of the same cell or cell-line.

20

25 64. A kit for determining whether a compound is an inhibitor or an enhancer of transcription of an unc-53 gene of C. elegans or a functional fragment of said gene which kit comprises at least a plurality of transgenic cells as claimed in Claims 52 or 53 and means for monitoring the reporter molecule.

30

65. A kit for determining whether a compound is an enhancer or an inhibitor of the activity of UNC-53 protein or a functional equivalent, derivative, fragment or bioprecursor of said protein, which kit

comprises at least, one mutant organism of C. elegans as claimed in claim 10 or a transgenic organism as claimed in any of claims 16 to 18 and a wild type organism of C. elegans.

5

66. An oligonucleotide probe which comprises the carboxy-terminal 1.5 kb of the coding nucleic acid sequence shown in Figure 1 or a fragment thereof comprising between 18 and 24 base pairs.

10

67. An oligonucleotide probe comprising a nucleic acid sequence encoding the amino acid sequence as numbered 1 to 110, 114 to 133, 487 to 495, 537 to 545, 1032 to 1037, 1097 to 1116 or 1300 to 1307 shown in Figure 3 or a fragment thereof.

15

68. A probe as claimed in Claim 66 or 67 which is labelled for detection.

20

69. A method of identifying homologues of a C. elegans unc-53 gene or a functional fragment thereof which method comprises hybridizing to a C. elegans DNA library an oligonucleotide probe as claimed in any one of Claims 66 to 68 under appropriate conditions of stringency to identify genes having statistically significant homology with the cDNA of any one of Claims 1 to 10.

25

70. A method of identifying a protein which is active in the signal transduction pathway of a cell of which an UNC-53 protein or a functional equivalent, fragment or bioprecursor of said UNC-53 protein is a component, which method comprises:

30

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component, which method comprises:

- 5 (a) contacting an extract of said cell with an antibody to the UNC-53 protein of C.elegans or a functional equivalent, fragment, derivative or bioprecursor of said protein,
- (b) identifying the antibody/UNC-53 complex, and
- 10 (c) analysing the complex to identify any protein bound to the UNC-53 protein other than the antibody.

71. A method of identifying a further protein which is active in the signal transduction pathway of a cell of which an UNC-53 protein or a functional equivalent, fragment or bioprecursor of said UNC-53 protein is a component which method comprises:

- (a) forming an antibody to the identified protein bound to the UNC-53 protein in Claim 65,
- 20 (b) contacting a cell extract with said antibody and identifying the antibody/protein complex,
- (c) analysing the complex to identify any further protein bound to the first protein other than the antibody, and
- 25 (d) optionally repeating steps (a) to (c) to identify further proteins in said pathway.

72. A method of identifying a protein which is active in the signal transduction pathway of a cell of which an UNC-53 protein or a functional equivalent, fragment or bioprecursor of said UNC-53 protein is a component, which method comprises

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- (a) contacting an extract of said cell with UNC-53 protein of C. elegans or a functional equivalent, derivative or bioprecursor of said UNC-53 protein
- 5 (b) identifying UNC-53 protein/protein complex formed and
- (c) analysing the complex to identify any protein bound to the UNC-53 protein other than another UNC-53 protein.

10

73. A method according to claim 72 which further comprises contacting a cell extract with any protein identified from step (c) not being UNC-53 protein and repeating steps (b) and (c) so as to identify any  
15 further protein involved in the signal transduction pathway of said cell.

74. A method of identifying a protein involved in the signal transduction pathway of C. elegans which  
20 method comprises:

- (a) constructing at least two nucleotide vectors, the first of which comprises a nucleotide segment encoding for a DNA binding domain of GAL4 protein fused to a sequence  
25 encoding UNC-53 protein of C. elegans or a functional equivalent, derivative, fragment or bioprecursor thereof, the second vector comprising a nucleotide sequence encoding a protein binding domain of GAL4 protein fused to a nucleotide sequence encoding a protein to be  
30 tested,
- (b) co-transforming each of said vectors into a yeast cell being deficient for transcription of genes encoding galactose metabolites, wherein

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interaction between said test protein and said UNC-53 protein leads to transcription of said galactose metabolite genes.

- 5      75. A protein identified by the method, of any one of claims 70 to 74 for use as a medicament to promote neuronal regeneration, revascularisation or wound healing, or for treatment of chronic neurodegenerative diseases or acute traumatic injuries.
- 10
76. Use of a protein identified by the methods of any one of claims 70 to 74 in the manufacture of a medicament for promoting neuronal regeneration, revascularisation or wound healing, or for treatment
- 15 of chronic neurodegenerative diseases or acute traumatic injuries.
77. A pharmaceutical composition comprising a protein identified by the methods of any one of Claims
- 20 70 to 74 and an acceptable carrier diluent, or excipient therefor.
78. A process for producing an UNC-53 protein of C. elegans or a functional equivalent fragment,
- 25 derivative or bioprecursor of said UNC-53 protein which process comprises culturing the transfected or transformed cells of Claim 12 or Claim 13 and recovering the expressed UNC-53 protein.
- 30 79. A process for producing an UNC-53 protein of C. elegans or a functional equivalent fragment, derivative or bioprecursor of said protein which process comprises culturing an insect cell transfected

with a recombinant Baculovirus vector, said vector comprising a DNA insert encoding said UNC-53 protein or a functional equivalent, fragment or bioprecursor thereof, downstream of the Baculovirus polyhedrin promoter, and recovering the expressed UNC-53 protein.

80. A hybridoma cell line deposited under the LMBP Accession No. 1383CB.

81. Monoclonal antibody 16-48-2 obtainable from the hybridoma deposited under the LMBP Accession No. 1383CB.

82. Plasmid pTB54 deposited under the LMBP Accession No. 3296.

83. Plasmid pBT112 deposited under the Accession No. 3295.

84. Plasmid pTB72 deposited under the LMBP Accession No. 3486.

85. Transgenic cell-line of C.elegans designated TB4EX25 and deposited under the LMBP Accession No. 1384CB.

86. Transgenic cell-line of C. elegans designated TBAIn76 and deposited under the Accession No. 1385CB.

87. A transgenic cell-line of MCF-7 breast carcinoma

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cells deposited under the LMBP Accession No. 1550CB.

88. A transgenic cell-line of N4 neuroblastoma  
cells deposited under LMBP Accession No. 1549CB.



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FIG. 1

TB6 & TB3

BSP1286

GGTTTAATTACCCAAGTTTGAGACATCAATTCCATCGAACGAAATGTTGGTGCTCCGA AT  
 10 20 30 40 50 60  
 OUT OF FRAME ATG

TTHIIII  
 .AHAI  
 .. AATII

BANI

AAAATGACGACGTCAAATGTAGAATTGATACCAATCTACACGGATTGGGCCAATCGGC AC  
 70 80 90 100 110 120

M T T S N V E L I P I Y T D W A N R H  
 ATG1

ASUII BBVI NRUI

CTTTCGAAGGGCAGCTTATCAAAGTCGATTAGGGATATTTCCAATGATTTTCGCGACT AT  
 130 140 150 160 170 180

L S K G S L S K S I R D I S N D F R D Y

TB1B

ECORI BSMI

CGACTGGTTTCTCAGCTTATTAATGTGATCGTTCCGATCAACGAATTCTCGCCTGCAT TC  
 190 200 210 220 230 240

R L V S Q L I N V I V P I N E F S P A F

TB16

AFLIII  
 FOKI

ACGAAACGTTTGGCAAAAATCACATCGAACCTGGATGGCCTCGAAACGTGTCTCGACT AC  
 250 260 270 280 290 300

T K R L A K I T S N L D G L E T C L D Y

TB1

HPHI ECORV NSPBII

CTGAAAAATCTGGGTCTCGACTGCTCGAAACTCACCAAAACCGATATCGACAGCGGAA AC  
 310 320 330 340 350 360

L K N L G L D C S K L T K T D I D S G N

BBVI MBOII  
 . NSPBII  
 . PVUII

HINDIII

TTGGGTGCAGTTCTCCAGCTGCTCTTCTGCTCTCCACCTACAAGCAGAAGCTTCGGC AA  
 370 380 390 400 410 420

L G A V L Q L L F L L S T Y K Q K L R Q

FOKI

MBOII NSPBII  
 .SACII

CTGAAAAAGATCAGAAGAAATTGGAGCAACTACCCACATCCATTATGCCACCCGCGG TT  
 430 440 450 460 470 480

L K K D Q K K L E Q L P T S I M P P A V  
 ATG 2

AFLIII

TCTAAATTACCCTCGCCACGTGTCGCCACGTGAGCAACCGCTTCAGCAACTAACCCTAA AT  
 490 500 510 520 530 540

S K L P S P R V A T S A T A S A T N P N

FOKI HINCII BSTNI

TCCAACCTTTCCACAAATGTCAACATCCAGGCTTCAGACTCCACAGTCAAGAATATCGA AA  
 550 560 570 580 590 600

S N F P Q M S T S R L Q T P Q S R I S K  
 ATG3

FIG. 1 CONTINUED.

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TB6B  
 |  
 ATTGATTCATCAAAGATTGGTATCAAGCCAAAGACGTCTGGACTTAAACCACCCTCAT CA  
 610 620 630 640 650 660  
 I D S S K I G I K P K T S G L K P P S S

AHAI  
 AATII  
 TCAACCACTTCATCAAATAATACAAATTCATTCCGTCGTCGAGCCGTTTCGAGTGGCA AT  
 670 680 690 700 710 720  
 S T T S S N N T N S F R P S S R S S G N

ECORV  
 MBOII  
 AATAATGTTGGCTCGACGATATCCACATCTGCGAAGAGCTTAGAATCATCATCAACGT AC  
 730 740 750 760 770 780  
 N N V G S T I S T S A K S L E S S S T Y

ASUII  
 XBAI  
 AGCTCTATTTTGAATCTAAACCGACCTACCTCCCAACTCCAAAAACCTTCTAGACCAC AA  
 790 800 810 820 830 840  
 S S I S N L N R P T S Q L Q K P S R P Q

NHEI  
 ACCCAGCTAGTTCGTGTTGCTACAACCTACAAAAATCGGAAGCTCAAAGCTAGCCGCTC CG  
 850 860 870 880 890 900  
 T Q L V R V A T T T K I G S S K L A A P

BSP1286  
 HGIAI  
 MBOII  
 BANII  
 BSP1286  
 AAAGCCGTGAGCACCCCAAACTTGCTTCTGTGAAGACTATTGGAGCAAAACAAGAGC CC  
 910 920 930 940 950 960  
 K A V S T P K L A S V K T I G A K Q E P

NSPBII  
 BSMI  
 MBOII  
 GATAACAGCGGTGGTGGTGGTGGTGGGAATGCTGAAATTAAAGTTATTTCAGTAGCAAAA AC  
 970 980 990 1000 1010 1020  
 D N S G G G G G G M L K L K L F S S K N  
 ATG4

BANI  
 CCATCTTCCTCATCGAATAGCCCAACCTACGAGAAAGGCGGCGGCTGCCTCAAC AA  
 1030 1040 1050 1060 1070 1080  
 P S S S S N S P Q P T R K A A A V P Q Q

BBVI  
 CAAACTTTGTGCGAAATCGCTGCCCCAGTGAAAAGTGGCCTGAAGCCGCCGACCAGTA AG  
 1090 1100 1110 1120 1130 1140  
 Q T L S K I A A P V K S G L K P P T S K

TB22  
 BSTXI  
 HINDIII  
 |  
 CTGGGAAGTGCCACGTCTATGTGCGAAGCTTTGTACGCCAAAAGTTTCTACCGTAAAA CG  
 1150 1160 1170 1180 1190 1200  
 L G S A T S M S K L C T P K V S Y R K T

AHAI  
 HGAI  
 SFANI  
 GACGCCCAATCATATCTCAACAAGACTCGAAACGATGCTCAAAGAGCAGTGAAGAAG AG  
 1210 1220 1230 1240 1250 1260  
 D A P I I S Q Q D S K R C S K S S E E E

FIG. 1 CONTINUED.

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MBOII  
 .BSPMII  
 ... MBOII  
 TCCGGATACGCTGGATTCAACAGCACGTCGCCAACGTCATCATCGACGGAAGGTTCCC TA  
 1270 1280 1290 1300 1310 1320  
 S G Y A G F N S T S P T S S S T E G S L

BSMI  
 SPHI  
 . MBOII  
 . NSII  
 AGCATGCATTCCACATCTTCCAAGAGTTCAACGTCAGACGAAAAGTCTCCGTCATCAG AC  
 1330 1340 1350 1360 1370 1380  
 S M H S T S S K S S T S D E K S P S S D  
 ATG5

GATCTTACTCTTAACGCCTCCATCGTGACAGCTATCAGACAGCCGATAGCCGCAACAC CG  
 1390 1400 1410 1420 1430 1440  
 D L T L N A S I V T A I R Q P I A A T P

SSPI  
 GTTCTCCAAATATTATCAACAAGCCTGTTGAGGAAAAACCAACTGGCAGTGAAAAG GA  
 1450 1460 1470 1480 1490 1500  
 V S P N I I N K P V E E K P T L A V K G

BINI XHOII NSPBII  
 PVUII  
 GTGAAAAGCACAGCGAAAAAAGATCCACCTCCAGCTGTTCCGCCACGTGACACCCAGC CA  
 1510 1520 1530 1540 1550 1560  
 V K S T A K K D P P P A V P P R D T Q P

HINCII ECORV  
 ACAATCGGAGTTGTTAGTCCAATTATGGCACATAAGAAGTTGACAAATGACCCCGTGA TA  
 1570 1580 1590 1600 1610 1620  
 T I G V V S P I M A H K K L T N D P V I

SFANI  
 TCTGAAAAACCGAACCTGAAAAGCTCCAATCAATGAGCATCGACACGACGGACGTTT CA  
 1630 1640 1650 1660 1670 1680  
 S E K P E P E K L Q S M S I D T T D V P

CCGCTTCCACCTCTAAAATCAGTTGTTCCACTTAAAATGACTTCAATCCGACAACCAC CA  
 1690 1700 1710 1720 1730 1740  
 P L P P L K S V V P L K M T S I R Q P P

MBOII  
 ACGTACGATGTTCTTCTAAAACAAGGAAAAATCACATCGCCTGTCAAGTCGTTTGGAT AT  
 1750 1760 1770 1780 1790 1800  
 T Y D V L L K Q G K I T S P V K S F G Y

HGAI HGAI  
 . MBOII  
 GAGCAGTCGTCCGCGTCTGAAGACTCCATTGTGGCTCATGCGTCGGCTCAGGTGACTC CG  
 1810 1820 1830 1840 1850 1860  
 E Q S S A S E D S I V A H A S A Q V T P

HPHI FOKI  
 CCGACAAAACTTCTGTAATCATTCGCTGGAGAGAAGGATGGGAAAGAATAAGACAT CA  
 1870 1880 1890 1900 1910 1920  
 P T K T S G N H S L E R R M G K N K T S

NSPBII AHAI HGAI  
 GAATCCAGCGGCTACACCTCTGACGCCGGTGTTCGATGTGCGCCAAAATGAGGGAGA AG

FIG. 1 CONTINUED. 4/99

NSPBII                      AHAI                      HGAII  
GAATCCAGCGCTACACCTCTGACGCCGGTGTGCGATGTGCGCCAAAATGAGGGAGAAG  
1930                      1940                      1950                      1960                      1970                      1980  
E S S G Y T S D A G V A M C A K M R E K

BSP1286  
HGIAI                      ASUII  
CTGAAAGAATACGATGACATGACTCGTCGAGCACAGAACGGCTATCCTGACAACTTCGAA  
1990                      2000                      2010                      2020                      2030                      2040  
L K E Y D D M T R R A Q N G Y P D N F E

MBOII                      BANII  
.                      BSP1286  
.                      HGIAI  
.                      SACI  
GACAGTTCCTCCTTGTCTGGAATATCCGATAACAACGAGCTCGACGACATATCCACG  
2050                      2060                      2070                      2080                      2090                      2100  
D S S S L S S G I S D N N E L D D I S T

BSPMII                      FOKI  
.                      ACCI  
GACGATTTGTCCGGAGTAGACATGGCAACAGTCGCCCTCCAAACATAGCGACTATTCCCAC  
2110                      2120                      2130                      2140                      2150                      2160  
D D L S G V D M A T V A S K E S D Y S H

MBOII                      AVAI                      AVAII  
.                      MBOII  
TTTGTTCGCCATCCCACGTCTTCTTCCTCAAAGCCCCGAGTCCCCAGTCGGTCCCTCCACA  
2170                      2180                      2190                      2200                      2210                      2220  
F V R E P T S S S S K P R V P S R S S T

AVAI  
XHOI  
TCAGTCGATTCTCGATCTCGAGCAGAACAGGAGAATGTGTACAACTTCTGTCCCGAGTC  
2230                      2240                      2250                      2260                      2270                      2280  
S V D S R S R A E Q E N V Y K L L S Q C

BBVI BGLI  
.                      BANI  
.                      .AHAI  
.                      .NARI  
.                      .BAEII  
.                      .NSPBII                      BINI XHOII  
.                      .FOKI  
CGAACGAGCCAACGTGGCGCGCTGCCACCTCAACCTTCGGACAACATTCCGCTAAGATCC  
2290                      2300                      2310                      2320                      2330                      2340  
R T S Q R G A A A T S T F G Q E S L R S

AVAI  
.NCII  
..NCII  
..SMAI  
. . . . . NSPBII  
. . . . . PVUII  
CCGGGATACTCATCTATTCTCCACACTTATCAGTGTGACGTGATAAGGACACAATGTCT  
2350                      2360                      2370                      2380                      2390                      2400  
P G Y S S Y S P E L S V S A D K D T M S

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FIG. 1 CONTINUED.

SPEI  
 . SALI  
 . .ACCI  
 . .HINCII  
 . .MBOII  
 ATGCACTCACAGACTAGTCGACGACCTTCTTCACAAAAACCAAGCTATTCAGGCCAAT TT  
 2410 2420 2430 2440 2450 2460  
 M H S Q T S R R P S S Q K P S Y S G Q F

FOKI BSP1286  
 HGIAI  
 CATTCACTTGATCGTAAATGCCACCTTCAAGAGTTCACATCCACCGAGCACAGAATGG CG  
 2470 2480 2490 2500 2510 2520  
 H S L D R K C H L Q E F T S T E H R M A

AVAI  
 .BANII  
 .BSP1286 BANI MBOII BINI BAMHI  
 XHOII  
 GCTCTCTTGAGCCCGAGACGGGTGCCGAACCTCGATGTCGAAATATGATTCTTCAGGAT CC  
 2530 2540 2550 2560 2570 2580  
 A L L S P R R V P N S M S K Y D S S G S

BINI AVAI  
 TACTCGGCGCGTTCCCCGAGGTGGAAGCTCTACTGGTATCTATGGAGAGACGTTCCAAC TG  
 2590 2600 2610 2620 2630 2640  
 Y S A R S R G G S S T G I Y G E T F Q L

BINI BAMHI  
 XHOII  
 CACAGACTATCCGATGAAAAATCCCCCGCACATTCTGCCAAAAGTGAGATGGGATCCC AA  
 2650 2660 2670 2680 2690 2700  
 H R L S D E K S P A H S A K S E M G S Q

BINI NHEI NDEI  
 XHOII BINI  
 CTATCACTGGCTAGCACGACAGCATATGGATCTCTCAATGAGAAGTACGAACATGCTA TT  
 2710 2720 2730 2740 2750 2760  
 L S L A S T T A Y G S L N E K Y E H A I

SALI  
 .ACCI  
 .HINCII  
 CGGGACATGGCACGTGACTTGGAGTGTTACAAGAACACTGTCGACTCACTAACCAAGA AA  
 2770 2780 2790 2800 2810 2820  
 R D M A R D L E C Y K N T V D S L T K K

HINDIII  
 CAGGAGAACTATGGAGCATTGTTTGATCTTTTTGAGCAAAAGCTTAGAAAACTCACTC AA  
 2830 2840 2850 2860 2870 2880  
 Q E N Y G A L F D L F E Q K L R K L T Q

BINI  
 CLAI MBOII  
 CACATTGATCGATCCAACTTGAAGCCTGAAGAGGCAATACGATTTCAGGCAGGACATTG CT  
 2890 2900 2910 2920 2930 2940  
 H I D R S N L K P E E A I R F R Q D I A

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FIG. 1 CONTINUED.

FOKI  
 . SFANI  
 CATTTGAGGGATATTAGCAATCATCTTGCATCCAACCTCAGCTCATGCTAACGAAGGCG CT  
 2950 2960 2970 2980 2990 3000  
 H L R D I S N H L A S N S A H A N E G A

MBOII HPHI  
 . HINCII FOKI  
 . SFANI CLAI CLAI  
 GGTGAGCTTCTTCGTCAACCATCTCTGGAATCAGTTGCATCCCATCGATCATCGATGT CA  
 3010 3020 3030 3040 3050 3060  
 G E L L R Q P S L E S V A S H R S S M S

ECOB BBVI MBOII  
 . BANII  
 . BSP1286  
 . HGIAI  
 . SACI  
 TCGTCGTCGAAAAGCAGCAAGCAGGAGAAGATCAGCTTGAGCTCGTTTGGCAAGAACA AG  
 3070 3080 3090 3100 3110 3120  
 S S S K S S K Q E K I S L S S F G K N K

BINI BAMHI  
 . XHOII  
 . MBOII  
 . BINI HPHI MBOII  
 . MBOII  
 AAGAGCTGGATCCGCTCCTCACTCTCCAAGTTCACCAAGAAGAACAAGAACTACG AC  
 3130 3140 3150 3160 3170 3180  
 K S W I R S S L S K F T K K K N K N Y D

NDEI XHOII  
 . BSPMII BINI  
 GAAGCACATATGCCATCAATTTCCGGATCTCAAGGAACCTTGACAACATTGATGTGA TT  
 3190 3200 3210 3220 3230 3240  
 E A H M P S I S G S Q G T L D N I D V I

BANII  
 BSP1286  
 HGIAI  
 SACI ECOK APALI  
 . BSP1286  
 . HGIAI  
 GAGTTGAAGCAAGAGCTCAAAGAACGCGATAGTGCACTTTACGAAGTCCGCCTTGACA AT  
 3250 3260 3270 3280 3290 3300  
 E L K Q E L K E R D S A L Y E V R L D N

BINI  
 . BSP1286  
 CTGGATCGTGCCCGCGAAGTTGATGTTCTGAGGGAGACAGTGAACAAGTTGAAAACCG AG  
 3310 3320 3330 3340 3350 3360  
 L D R A R E V D V L R E T V N K L K T E

HPHI AVAII MBOII  
 AACAAAGCAATTAAAGAAAGAAGTGACAAACTCACCAACGGTCCAGCCACTCGTGCTT CT  
 3370 3380 3390 3400 3410 3420  
 N K Q L K K E V D K L T N G P A T R A S

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SFANI  
 TCCCGCGCCTCAATTCCAGTTATCTACGACGATGAGCATGTCTATGATGCAGCGTGTA GC  
 3430 3440 3450 3460 3470 3480  
 S R A S I P V I Y D D E H V Y D A A C S  
 BBVI MBOII ASUII  
 . BINI  
 .. BBVI  
 AGTACATCAGCTAGTCAATCTTCGAAACGATCCTCTGGCTGCAACTCAATCAAGGTTA CT  
 3490 3500 3510 3520 3530 3540  
 S T S A S Q S S K R S S G C N S I K V T  
 PVUI  
 . HINCII  
 . HPAI  
 . NCII  
 GTAAACGTGGACATCGCTGGAGAAATCAGTTCGATCGTTAACCCGGACAAAGAGATAA TC  
 3550 3560 3570 3580 3590 3600  
 V N V D I A G E I S S I V N P D K E I I  
 ECVR HINCII  
 GTAGGATATCTTGCCATGTCAACCAGTCAGTCATGCTGGAAAGACATTGATGTTTCTA TT  
 3610 3620 3630 3640 3650 3660  
 V G Y L A M S T S Q S C W K D I D V S I  
 ACCI SFANI CLAI  
 CTAGGACTATTTGAAGTCTACCTATCCAGAATTGATGTGGAGCATCAACTTGGAAATCG AT  
 3670 3680 3690 3700 3710 3720  
 L G L F E V Y L S R I D V E H Q L G I D  
 SFANI STYI HGAI AFLIII  
 . MLUI  
 .HPHI HGAI  
 GCTCGTGATTCTATCCTTGGCTATCAAATTGGTGAACCTTCGACGCGTCATTGGAGACT CC  
 3730 3740 3750 3760 3770 3780  
 A R D S I L G Y Q I G E L R R V I G D S  
 FOKI  
 ACAACCATGATAACCAGCCATCCAACCTGACATTCTTACTTCCTCAACTACAATCCGAA TG  
 3790 3800 3810 3820 3830 3840  
 T T M I T S H P T D I L T S S T T I R M  
 BANI ACCI AVAII MBOII  
 TTCATGCACGGTGCCGCACAGAGTCGCGTAGACAGTCTGGTCCTTGATATGCTTCTTC CA  
 3850 3860 3870 3880 3890 3900  
 F M H G A A Q S R V D S L V L D M L L P  
 AHAI  
 . AATII  
 AAGCAAATGATTCTCCAACCTCGTCAAGTCAATTTTGACAGAGAGACGTCTGGTGTTAG CT  
 3910 3920 3930 3940 3950 3960  
 K Q M I L Q L V K S I L T E R R L V L A  
 BBVI BSTNI  
 MBOII  
 GGAGCAACTGGAATTGGAAGAGCAAACCTGGCGAAGACCCTGGCTGCTTATGTATCTA TT  
 3970 3980 3990 4000 4010 4020  
 G A T G I G K S K L A K T L A A Y V S I

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FIG. 1 CONTINUED. 9/99

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BINI BAMHI
. XHOII BINI TTHIIII EAEI NCII
CTTCGAGGATCCCACCGACATCGTCTCTAAAAAATGGCCGTGGTTTCGATGGTGAAAAC CC
4630 4640 4650 4660 4670 4680
L R G S H R H R L

HPHI MBOII
. .BSP1286
. .HGIAI TTHIIII
. .HPHI FOKI BSPMI
GGAGAATGTGCTCAAACGTCTTCAACTCCAAGACCTCGTCCCGTCACCTGCCAACTCA TC
4690 4700 4710 4720 4730 4740

AVAI
XHOI BINI SFANI
. SPHI
CCGACAACACTTCAATCCCCTCGAGTCGTTGATCCAATTGCATGCTACCAAGCATCAG AC
4750 4760 4770 4780 4790 4800

MBOII MBOII MBOII
CATCGACAACATTTGAACAGAAGACTCTAATCTTCTCTCGCCTCTCCCCCGCTTTCCT TA
4810 4820 4830 4840 4850 4860

BANI
. KPNI
TCTTCGTACCGGTACCTGATGATTCCCCATTTCCCCCTTTTCCCCCAATTTCCCAG AA
4870 4880 4890 4900 4910 4920

AVAI
. NCII
. .NCII
. .SMAI
. . . BANI AHAI HGAI DRAI
CCTCCTGTTCCCTTTGTTCTAGTCCTCCCGGTGCCGACGCCGAAGCGATTAAAAA CC
4930 4940 4950 4960 4970 4980

XMNI
TTTTTCTTTCCGAAACATTTCCCATGCTCATTAAATAGTCAAATTGAATAAACAGTGT AT
4990 5000 5010 5020 5030 5040

GTACTTAAAAAAAAAAAAAAAAAAAAAAAAAAAA
5050 5060 5070

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## COMPARISON OF 7A VS 8A CLONE

10/99 FIG. 2

TB6 & TB3  
 BSP1286  
 BGIAI  
 GGTTTAATTACCCAAGTTTGAGACATCAATTCCATCGAACGAAATGTTGGTGCTCCGAAT  
 10 20 30 40 50 60  
 TTHIII  
 .AHAI  
 .. AATII  
 AAAATGACGACGTCAAATGTAGAATTGATACCAATCTACACGGATTGGGCCAATCGGCAC  
 70 80 90 100 110 120  
 M T T S N V E L I P I Y T D W A N R E  
 ASUII BBVI NRUI  
 CTTTCGAAGGGCAGCTTATCAAAGTCGATTAGGGATATTTCCAATGATTTTCGCGACTAT  
 130 140 150 160 170 180  
 L S K G S L S K S I R D I S N D F R D Y  
 TB1B  
 ECORI BSMI  
 CGACTGGTTTCTCAGCTTATTAATGTGATCGTTCCGATCAACGAATTCTCGCCTGCATTC  
 190 200 210 220 230 240  
 R L V S Q L I N V I V P I N E F S P A F  
 TB16  
 BSTNI AFLIII  
 FOKI  
 ACGAAACGTTTGGCAAAATCACATCGAACCTGGATGGCCTCGAAACGTGTCTCGACTAC  
 250 260 270 280 290 300  
 T K R L A K I T S N L D G L E T C L D Y  
 TB1  
 HPBI ECORV NSPBII  
 CTGAAAAATCTGGGTCTCGACTGCTCGAAACTCACCAAACCGATATCGACAGCGGAAAC  
 310 320 330 340 350 360  
 L K N L G L D C S K L T K T D I D S G N  
 BBVI MBOII  
 . NSPBII  
 . PVUII  
 HINDIII  
 TTGGGTGCAGTTCTCCAGCTGCTCTTCTCTCCACCTACAAGCAGAAGCTTCGGCAA  
 370 380 390 400 410 420  
 L G A V L Q L L F L L S T Y K Q K L R Q  
 FOKI  
 . MBOII NSPBII  
 . SACII  
 CTGAAAAAAGATCAGAAGAAATTGGAGCAACTACCCACATCCATTATGCCACCCGCGGTT  
 430 440 450 460 470 480  
 L K K D Q K K L E Q L P T S I M P P A V  
 AFLIII  
 TCTAAATTACCCTCGCCACGTGTGCGCCACGTCAGCAACCGCTTCAGCAACTAACCCTAAAT  
 490 500 510 520 530 540  
 S K L P S P R V A T S A T A S A T N P N  
 FOKI HINCII BSTNI  
 TCCAACCTTTCCACAAATGTCAACATCCAGGCTTCAGACTCCACAGTCAAGAATATCGAAA  
 550 560 570 580 590 600  
 S N F P Q M S T S R L Q T P Q S R I S K

FIG. 2 CONTINUED.

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**TB6B**  
 ATTGATTCATCAAAGATTGGTATCAAGCCAAAGACGTCTGGACTTAAACCACCCTCATCA  
 610 620 630 640 650 660  
 I D S S K I G I K P K T S G L K P P S S

**ABAI**  
**AATII**  
 TCAACCACTTCATCAAATAATACAAATTCATTCCGTCGAGCCGTTTCGAGTGGCAAT  
 670 680 690 700 710 720  
 S T T S S N N T N S F R P S S R S S G N

**ECORV**  
**MBOII**  
 AATAATGTTGGCTCGACGATATCCACATCTGCGAAGAGCTTAGAATCATCATCAACGTAC  
 730 740 750 760 770 780  
 N N V G S T I S T S A K S L E S S S T Y

**ASUII**  
**XBAI**  
 AGCTCTATTTTGAATCTAAACCGACCTACCTCCCAACTCCAAAAACCTTCTAGACCACAA  
 790 800 810 820 830 840  
 S S I S N L N R P T S Q L Q K P S R P Q

**NBEI**  
 ACCCAGCTAGTTTCGTGTTGCTACAACCTACAAAAATCGGAAGCTCAAAGCTAGCCGCTCCG  
 850 860 870 880 890 900  
 T Q L V R V A T T T K I G S S K L A A P

**BSP1286**  
**HGIAI**  
**MBOII**  
**BANII**  
**BSP1286**  
 AAAGCCGTGAGCACCCCAAACTTGCTTCTGTGAAGACTATTGGAGCAAAACAAGAGCCC  
 910 920 930 940 950 960  
 K A V S T P K L A S V K T I G A K Q E P

**NSPBII**  
**BSMI**  
**MBOII**  
 GATAACAGCGGTGGTGGTGGTGGTGGTGAATGCTGAAATTAAAGTTATTTCAGTAGCAAAAAAC  
 970 980 990 1000 1010 1020  
 D N S G G G G G G M L K L K L F S S K N

**BANI**  
 CCATCTTCTCATCGAATAGCCCACAACCTACGAGAAAGGCGGCGGCGGTGCCTCAACAA  
 1030 1040 1050 1060 1070 1080  
 P S S S S N S P Q P T R K A A A V P Q Q

**BBVI**  
 CAACTTTGTGCGAAAATCGCTGCCCCAGTGAAAAGTGGCCTGAAGCCGCGGACCAAGTAAG  
 1090 1100 1110 1120 1130 1140  
 Q T L S K I A A P V K S G L K P P T S K

**TB22**  
**BSTXI**  
**HINDIII**  
 CTGGGAAGTGCCACGTCTATGTGGAAGCTTTGTACGCCAAAAGTTTCCTACCGTAAAACG  
 1150 1160 1170 1180 1190 1200  
 L G S A T S M S K L C T P K V S Y R K T

**ABAI** **HGAI** **SFANI**  
 GACGCCCCAATCATATCTCAACAAGACTCGAAACGATGCTCAAAGAGCAGTGAAGAAGAG  
 1210 1220 1230 1240 1250 1260  
 D A P I I S Q Q D S K R C S K S S E E E

FIG. 2 CONTINUED.

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MBOII  
 .BSPMII  
 .. MBOII  
 TCCGATACGCTGGATTCAACAGCACGTGCGCAACGTCATCATCGACGGAAGGTTCCCTA  
 1270 1280 1290 1300 1310 1320  
 S G Y A G F N S T S P T S S S T E G S L

BSMI  
 SPHI  
 . MBOII  
 . NSII | START CE7  
 AGCATGCATTCCACATCTTCCAAGAGTTCAACGTCAGACGAAAAGTCTCCGTCATCAGAC  
 1330 1340 1350 1360 1370 1380  
 S M H S T S S K S S T S D E K S P S S D

GATCTTACTCTTAACGCCTCCATCGTGACAGCTATCAGACAGCCGATAGCCGCAACACCG  
 1390 1400 1410 1420 1430 1440  
 D L T L N A S I V T A I R Q P I A A T P

SSPI  
 GTTCTCCAAATATTATCAACAAGCCTGTTGAGGAAAAACCAACTGGCAGTGAAAGGA  
 1450 1460 1470 1480 1490 1500  
 V S P N I I N K P V E E K P T L A V K G

BINI XBOII NSPBII  
 PVUII  
 GTGAAAAGCACAGCGAAAAAAGATCCACCTCCAGCTGTTCCGCCACGTGACACCCAGCCA  
 1510 1520 1530 1540 1550 1560  
 V K S T A K K D P P P A V P P R D T Q P

BINCII ECORV  
 ACAATCGGAGTTGTTAGTCCAATTATGGCACATAAGAAGTTGACAAATGACCCCGTGATA  
 1570 1580 1590 1600 1610 1620  
 T I G V V S P I M A H K K L T N D P V I

SFANI  
 TCTGAAAAACCAGAACCTGAAAAGCTCCAATCAATGAGCATCGACACGACGGACGTTCCA  
 1630 1640 1650 1660 1670 1680  
 S E K P E P E K L Q S M S I D T T D V P

CCGCTTCCACCTCTAAAATCAGTTGTTCCACTTAAAATGACTTCAATCCGACAACCACCA  
 1690 1700 1710 1720 1730 1740  
 P L P P L K S V V P L K M T S I R Q P P

MBOII  
 ACGTACGATGTTCTTCTAAAACAAGGAAAAATCACATCGCCTGTCAAGTCGTTTGGATAT  
 1750 1760 1770 1780 1790 1800  
 T Y D V L L K Q G K I T S P V K S F G Y

HGAI HGAI  
 . MBOII  
 GAGCAGTCGTCCGCGTCTGAAGACTCCATTGTGGCTCATGCGTCGGCTCAGGTGACTCCG  
 1810 1820 1830 1840 1850 1860  
 E Q S S A S E D S I V A H A S A Q V T P

HPHI FOKI  
 CCGACAAAACCTTCTGGTAATCATTGCTGGAGAGAAGGATGGGAAAGAATAAGACATCA  
 1870 1880 1890 1900 1910 1920  
 P T K T S G N H S L E R R M G K N K T S

FIG. 2 CONTINUED.

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NSPBII					AHAI I					HGAI																			
GAATCCAGCGGCTACACCTCTGACGCCGGTGTTGCGATGTGCGCCAAAATGAGGGAGAAG																													
1930					1940					1950					1960					1970					1980				
E	S	S	G	Y	T	S	D	A	G	V	A	M	C	A	K	M	R	E	K										

BSP1286  
 HGIAI  
 ASUII  
 CTGAAAGAATACGATGACATGACTCGTCGAGCACAGAACGGCTATCCTGACAACTTCGAA  
 1990 2000 2010 2020 2030 2040  
 L K E Y D D M T R R A Q N G Y P D N F E

MBOII  
 .  
 .  
 .  
 BANII  
 BSP1286  
 HGIAI  
 SACI  
 GACAGTTCCTCTTGTCGTCTGGAATATCCGATAACAACGAGCTCGACGACATATCCACG  
 2050                      2060                      2070                      2080                      2090                      2100  
 D S S S L S S G I S D N N E L D D I S T

BSPMII  
 . ACCI  
 FOKI  
 GACGATTTGTCCGGAGTAGACATGGCAACAGTCGCTCCAAACATAGCGACTATTCCCAC  
 2110 2120 2130 2140 2150 2160  
 D D L S G V D M A T V A S K E S D Y S E

MBOII  
. MBOII AVAI AVAII  
TTTGTTCGCCATCCCACGCTCTTCTTCCTCAAAGCCCCGAGTCCCCAGTCGGTCTCTCCACA  
2170 2180 2190 2200 2210 2220  
F V R B P T S S S S K P R V P S R S S T

AVAI  
XHOI  
TCAGTCGATTCTCGATCTCGAGCAGAACAGGAGAATGTGTACAAACTTCTGTCCCAAGTGC  
2230 2240 2250 2260 2270 2280  
S V D S R S R A E Q E N V Y K L L S Q C

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BBVI BGLI
. . BANI
. . .ABAIL
. . .NARI
. . . .BAEII
. . . .NSPBII
. . . . .BINI XHOII
. . . . . .FOKI
CGAACGAGCCAAACGTGGCGCGCTGCCACCTCAACCTTCGGACAACATTTCGCTAAGATCC
2290          2300          2310          2320          2330          2340
R T S Q R G A A A T S T F G Q H S L R S

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AVAI
.NCII
..NCII
..SMAI
...
NSPBII
PVUII
CCGGGATACTCATCTATTCTCCCACTTATCAGTGTCAGCTGATAAGGACACAATGTCT
      2350      2360      2370      2380      2390      2400
P  G  Y  S  S  Y  S  P  H  L  S  V  S  A  D  K  D  T  M  S

```

FIG. 2 CONTINUED.

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SPEI  
 . . . SALI  
 . . . .ACCI  
 . . . .HINCII  
 . . . .MBOII  
 ATGCACTCACAGACTAGTCGACGACCTTCTTCACAAAACCAAGCTATTCAGGCCAATTT  
 2410 2420 2430 2440 2450 2460  
 M H S Q T S R R P S S Q K P S Y S G Q F

FOKI BSP1286  
 HGIAI  
 CATTCACTTGATCGTAAATGCCACCTTCAAGAGTTCACATCCACCGAGCACAGAATGGCG  
 2470 2480 2490 2500 2510 2520  
 H S L D R K C H L Q E F T S T E H R M A

AVAI  
 .BANII  
 .BSP1286 BANI MBOII BINI BAMHI  
 XHOII  
 GCTCTCTTGAGCCCGAGACGGGTGCCGAACCTCGATGTGCGAAATATGATTCTTCAGGATCC  
 2530 2540 2550 2560 2570 2580  
 A L L S P R R V P N S M S K Y D S S G S

BINI AVAI  
 TACTCGGCGCGTTCCCGAGGTGGAAGCTCTACTGGTATCTATGGAGAGACGTTCCAACGT  
 2590 2600 2610 2620 2630 2640  
 Y S A R S R G G S S T G I Y G E T F Q L

BINI BAMHI  
 XHOII  
 CACAGACTATCCGATGAAAAATCCCCGCACATTCTGCCAAAAGTGAGATGGGATCCCAA  
 2650 2660 2670 2680 2690 2700  
 H R L S D E K S P A H S A K S E M G S Q

BINI NHEI NDEI  
 XHOII BINI  
 CTATCACTGGCTAGCACGACAGCATATGGATCTCTCAATGAGAAGTACGAACATGCTATT  
 2710 2720 2730 2740 2750 2760  
 L S L A S T T A Y G S L N E K Y E H A I

SALI  
 .ACCI  
 .HINCII  
 CGGGACATGGCACGTGACTTGGAGTGTACAAGAACACTGTGCGACTCACTAACCAAGAAA  
 2770 2780 2790 2800 2810 2820  
 R D M A R D L E C Y K N T V D S L T K K

HINDIII  
 CAGGAGAACTATGGAGCATTGTTTGATCTTTTGGAGCAAAGCTTAGAAAACCTCACTCAA  
 2830 2840 2850 2860 2870 2880  
 Q E N Y G A L F D L F E Q K L R K L T Q

BINI  
 CLAI MBOII  
 CACATTGATCGATCCAACCTTGAAGCCTGAAGAGGCAATACGATTTCAGGCAGGACATTGCT  
 2890 2900 2910 2920 2930 2940  
 E I D R S N L K P E E A I R P R Q D I A

FIG. 2 CONTINUED.

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FOKI  
 . SFANI  
 CATTGAGGGATATTAGCAATCATCTTGCATCCAACCTCAGCTCATGCTAACGAAGGCGCT  
 2950 2960 2970 2980 2990 3000  
 H L R D I S N E L A S N S A H A N E G A

MBOII  
 . HPHI  
 . HINCII FOKI  
 . SFANI  
 . CLAI CLAI  
 GGTGAGCTTCTTCGTCAACCATCTCTGGAATCAGTTGCATCCCATCGATCATCGATGTCA  
 3010 3020 3030 3040 3050 3060  
 G E L L R Q P S L E S V A S H R S S M S

ECOB BBVI MBOII  
 . BANII  
 . BSP1286  
 . HGIAI  
 . SACI  
 TCGTCGTCGAAAAGCAGCAAGCAGGAGAAGATCAGCTTGAGCTCGTTTGGCAAGAACAAG  
 3070 3080 3090 3100 3110 3120  
 S S S K S S K Q E K I S L S S F G K N K

BINI BAMBI  
 . XHOII  
 . MBOII  
 . BINI HPHI  
 . MBOII  
 . MBOII  
 AAGAGCTGGATCCGCTCCTCACTCTCCAAGTTCACCAAGAAGAACAAGAACTACGAC  
 3130 3140 3150 3160 3170 3180  
 K S W I R S S L S K F T K K K N K N Y D

NDEI XHOII  
 . BSPMII BINI  
 GAAGCACATATGCCATCAATTTCCGGATCTCAAGGAACCTTTGACAACATTGATGTGATT  
 3190 3200 3210 3220 3230 3240  
 E A H M P S I S G S Q G T L D N I D V I

BANII  
 BSP1286  
 HGIAI  
 SACI ECOK APALI  
 . BSP1286  
 . HGIAI  
 GAGTTGAAGCAAGAGCTCAAAGAACGCGATAGTGCACCTTACGAAGTCCGCCTTGACAAT  
 3250 3260 3270 3280 3290 3300  
 E L K Q E L K E R D S A L Y E V R L D N

BINI  
 . BSP1286  
 CTGGATCGTGCCCGCGAAGTTGATGTTCTGAGGGAGACAGTGAACAAGTTGAAAACCGAG  
 3310 3320 3330 3340 3350 3360  
 L D R A R E V D V L R E T V N K L K T E

HPHI AVAII MBOII  
 AACAAAGCAATTAAAGAAAGAAGTGGACAAACTCACCAACGGTCCAGCCACTCGTGCTTCT  
 3370 3380 3390 3400 3410 3420  
 N K Q L K K E V D K L T N G P A T R A S

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BBVI	MBOII	ASUII
.	.	.BINI
.	.	.. BBVI

PVUI  
 . HINCII  
 . EPAI



FIG. 2 CONTINUED.

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ASUII | CE6 MBOII BSMI  
 CGAACAAATCAATCCGAAGATAGTATTGTTAATATCAGCATTCCTGAAAACAATAAGAA  
 4030 4040 4050 4060 4070 4080  
 R T N Q S E D S I V N I S I P E N N K E  
  
 XMNI MBOII AHAI  
 . . . . . BSTNI  
 . . . . . HGAI  
 . . . . . BGLII  
 . . . . . XBOII SFANI NSII  
 GAATTGCTTCAAGTGGACGACGCTGGAAAAGATCTTGAGAAGCAAAGAATCATGCATC  
 4090 4100 4110 4120 4130 4140  
 E L L Q V E R R L E K I L R S K E S C I  
  
 XBAI  
 GTAATTCTAGATAATATCCCAAAGAATCGAATTGCATTGTGTATCCGTTTTTGCAAAT  
 4150 4160 4170 4180 4190 4200  
 V I L D N I P K N R I A F V V S V F A N  
  
 AVAI HINCII ECORV  
 GTCCCACTTCAAACAACGAAGGTCCATTTGTAGTATGCACAGTCAACCGATATCAAATC  
 4210 4220 4230 4240 4250 4260  
 V P L Q N N E G P F V V C T V N R Y Q I  
  
 HPHI FOKI  
 CCTGAGCTTCAAATTCACCACAATTTCAAATGTGAGTAATGTGCAATCGTCTCGAAGGA  
 4270 4280 4290 4300 4310 4320  
 P E L Q I H H N F K M S V M S N R L E G  
  
 FOKI  
 TTCATCCTACGTTACCTCCGACGACGGGCGGTAGAGGATGAGTATCGTCTAACTGTACAG  
 4330 4340 4350 4360 4370 4380  
 F I L R Y L R R R A V E D E Y R L T V Q  
  
 MBOII  
 . SFANI  
 . . . . . BANII  
 . . . . . BSP1286  
 . . . . . HGIAI  
 . . . . . SACI MBOII MBOII  
 ATGCCATCAGAGCTCTTCAAATCATTGACTTCTTCCCAATAGCTCTTCAGGCCGTCAAT  
 4390 4400 4410 4420 4430 4440  
 M P S E L F K I I D F F P I A L Q A V N  
 ECOR1 USED FOR EXPRESSION  
 ECORI AVAI SPHI  
 AATTTTATTGAGAAAACGAATTCTGTTGATGTGACAGTTGGTCCAAGAGCATGCTTGAAC  
 4450 4460 4470 4480 4490 4500  
 N F I E K T N S V D V T V G P R A C L N  
  
 BINI BAMBI  
 . XBOII BINI  
 TGTCTCTAACTGTGATGGATCCCGTGAATGGTTCATTGCGATTGTGGAATGAGAACTTC  
 4510 4520 4530 4540 4550 4560  
 C P L T V D G S R E W F I R L W N E N F  
  
 AFLIII BBVI  
 ATTCCATATTTGGAACGTGTTGCTAGAGATGGCAAAAAAACCTTCGGTTCGCTGCACTTC  
 AAAAAA-ACC...  
 4570 4580 4590 4600 4610 4620  
 I P Y L E R V A R D G K K N L R S L H F  
 T F G R C T S

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## FIG. 2 CONTINUED.

BINI BAMHI  
 . XBOII BINI TTHIIII EAEI NCII  
 CTTTCGAGGATCCCACCGACATCGTCTCTAAAAAATGGCCGTGGTTCGATGGTGAAAACCC  
 4630 4640 4650 4660 4670 4680  
 L R G S H R H R L \*  
 F E D P T D I V S E K W P W F D G E N P  
 HPHI MBOII  
 . .BSP1286  
 . .BGIAI TTHIIII  
 . . . .BPHI FOKI BSPMI  
 GGAGAATGTGCTCAAACGTCTTCAACTCCAAGACCTCGTCCCGTCACCTGCCAACTCATC  
 4690 4700 4710 4720 4730 4740  
 E N V L K R L Q L Q D L V P S P A N S S  
  
 AVAI  
 XBOI BINI SFANI  
 . SPHI  
 CCGACAACACTTCAATCCCCCTCGAGTCGTTGATCCAATTGCATGCTACCAAGCATCAGAC  
  
 4750 4760 4770 4780 4790 4800  
 R Q H F N P L E S L I Q L H A T K H Q T  
 MBOII MBOII MBOII  
 CATCGACAACATTTGAACAGAAGACTCTAATCTTCTCTCGCCTCTCCCCCGCTTTCCTTA  
 4810 4820 4830 4840 4850 4860  
  
 I D N I \*  
 BANI  
 . KPNI  
 TCTTCGTACCGGTACCTGATGATTCCCCATTTTCCCCCTTTTCCCCCAATTTCCCAGAA  
 4870 4880 4890 4900 4910 4920  
  
 AVAI  
 .NCII  
 ..NCII  
 ..SMAI  
 ... BANI AHAI HGAI DRAI  
 CCTCCTGTTCCTTTGTTTCCTAGTCCTCCCGGTGCCGACGCCGAAGCGATTTAAAAACC  
 4930 4940 4950 4960 4970 4980  
  
 XMNI  
 TTTTCTTTCCGAAACATTTCCCATTTGCTCATTAATAGTCAAATTGAATAAACAGTGTAT  
 4990 5000 5010 5020 5030 5040  
  
 GTACTTAAAAAAAAAAAAAAAAAAAAAAAAA  
 5050 5060 5070

FIG. 3

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Sequences of low complexity in UNC-53 TB3-M5 identified with the FILTER and SEG algorithms of the BLAST sequence homology package.

MTTSNVELIPIYTDWANRHLSKGSLSKSIRDISNDFRDYRLVSQLINVIVPINEFSPAFT  
 KRLAKITSNLDGLETCLDYLNGLDCLTKTDIDSGNLGAVLQLLFLLSTYXXXXXXX  
 XXXXXXXXXXXXPTSIMPPAVSKLXXXXXXXXXXXXXXXXXXXXFPQMSTSRLOTPOQXXXXXX  
 XXXXXXXXXXXXTSGLKPKXX  
 XXXNLNRPTSQLQKPSRPQTQLVRVATTTKIGSSKLAAPKAVSTPKLASVKTIGAKQEPD  
 NSXXXXXXXXXXXXXXXXXXXXXXXXXXXXQPTRKAAAVPQQOTLSKIAAPVKSGLKPPPTSKL  
 GSATSMKLCPTPKVSYRKTDAPIIISQQDSKRCSSKXXXXXXGYAGFNXXXXXXXXXXXXXX  
 XXXXXXXXXXXXXXXXXXXXDDLTLNASIVTAIRQPIAATPVSPNIINKPVEEKPTLAVKGV  
 KSTAKKDPPPAVPPRDTQPTIGVVSPIMAHKKLTNDPVISEKPEPEKLQSMSIDTTDXXX  
 XXXXXXXXXXXXMTSIRQPPTYDVLLKQGKITSPVKSFQYEQSSASEDSIVAHASQVTPP  
 TKTSGNHSLERRMGKNKTSSESSGYTSDAGVAMCAKMKREKLKEYDDMTRRAQNGYPDNFED  
 XXXXXXXXXXXXNNELDDISTDDLSGVDMATVASKHSDYSHFVRHPXXXXXXXXXXXXXXXXXX  
 XXXXXAAEQENVYKLLSQCRTSQRGAAATSTFGQHSRLSPGYSSYSPHLSVSADKDTMSM  
 HSQTSRRPSSQKPSYSGQFHS�DRKCHLQFETSTEHRMAALLSPRRVPNXXXXXXXXXXXX  
 XXXXXXXXXXXXXIYGETFQLHRLSDEKSPAHSKSEMGSQSLASTTAYGSLNEKYEHAIR  
 DMARDLECYKNTVDLSLTKKQENYALFDLFEQKLRLKLTQHIDRSNLKPEEAIRFRQDIAH  
 LRDISNHLASNSAHANEGAGELLRQPSLEXXXXXXXXXXXXXXXXXXXXXXXXXXXXXFGKNKK  
 SWIRSSLSKFTKKKNKNYDEAHMPSISGSQGTLDNIDVIELKQELKERDSALYEVRLDNL  
 DRAREVDVLRETVNKLKTENKQKKKEVDKLTNGPATRASSRASIPVIYDDEHVYDXXXXXX  
 XXXXXXXXXXXXGVCNXXXXXXXXXXXXXXXXXXXXKEIIVGYLAMSTSQSCWKDIDVSL  
 GLFEVYLSRIDVEHQLGIDARDSILGYQIGELRRVIGDSTTMITSHPTDILTSSTTIRM  
 MHGAAQSRVDSLVDMLLPKQMIQLVKLSILTERRLVLAGATGIGKSKLAKTLAAVVSIR  
 TNQSEDSIVNISIPENNKEELLQVERRLEKILRSKESCIVILDNIPKNRIAFVVSFANV  
 PLQNNEGPFVCTVNRYQIPELQIHNFKMSVMSNRLEGFILRYLRRRAVEDEYRLTVQM  
 PSELFKIIDFFPIALQAVNNFIEKTSVDVTVGPRACLNCPLTVDGSREWFIRLWNNENFI  
 PYLERVARDGKKNLRLSLHLRGSRRHRL

MTTSNVELIPIYTDWANRHLSKGSLSKSIRDISNDFRDYRLVSQLINVIVPINEFSPAFT  
 KRLAKITSNLDGLETCLDYLNGLDCLTKTDIDSGNLGAVLQLLFLLSTYKOKLRQL  
 KKDOKKLEQLPTSIMPPAVSKLPSPRVATSATASATNPNSNFPQMSTSRLOTPOSRIKI  
 DSSKIGIKPKTSGLKPPSSSTTSNNNTNSFRPSSRSSGNNNVGSTISTSAKSLESSSTYS  
 SISNLNRPTSQLQKPSRPQTQLVRVATTTKIGSSKLAAPKAVSTPKLASVKTIGAKQEPD  
 NSGGGGGMLKLLFSSKNPSSSSNSPQPTRKAAAVPQQOTLSKIAAPVKSGLKPPPTSKL  
 GSATSMKLCPTPKVSYRKTDAPIIISQQDSKRCSSSEEEESGYAGFNSTSTSSSTEGSL  
 MHSTSSKSSTSDEKSPSSDDLTLNASIVTAIRQPIAATPVSPNIINKPVEEKPTLAVKGV  
 KSTAKKDPPPAVPPRDTQPTIGVVSPIMAHKKLTNDPVISEKPEPEKLQSMSIDTTDVP  
 LPPLKSUVPLKMTSIRQPPTYDVLLKQGKITSPVKSFQYEQSSASEDSIVAHASQVTPP  
 TKTSGNHSLERRMGKNKTSSESSGYTSDAGVAMCAKMKREKLKEYDDMTRRAQNGYPDNFED  
 SSSLSSGISDNNELDDISTDDLSGVDMATVASKHSDYSHFVRHPTSSSSKPRVPSRSSTS  
 VDSRSRAEQENVYKLLSQCRTSQRGAAATSTFGQHSRLSPGYSSYSPHLSVSADKDTMSM  
 HSQTSRRPSSQKPSYSGQFHS�DRKCHLQFETSTEHRMAALLSPRRVPNSMSKYDSSGSY  
 SARSRGGSSTGIYGETFQLHRLSDEKSPAHSKSEMGSQSLASTTAYGSLNEKYEHAIR  
 DMARDLECYKNTVDLSLTKKQENYALFDLFEQKLRLKLTQHIDRSNLKPEEAIRFRQDIAH  
 LRDISNHLASNSAHANEGAGELLRQPSLESVASHRSSMSSSSKSSKOEKISLSSFGKNKK  
 SWIRSSLSKFTKKKNKNYDEAHMPSISGSQGTLDNIDVIELKQELKERDSALYEVRLDNL  
 DRAREVDVLRETVNKLKTENKQKKKEVDKLTNGPATRASSRASIPVIYDDEHVYDAACSS

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FIG. 3 CONTINUED.

TSASOSSKRSSGCNSIKVTVNVDIAGEISSIVNPDKEIIVGYLAMSTSQSCWKDIDVSIL  
GLFEVYLSRIDVEHQLGIDARDSILGYQIGELRRVIGDSTTMITSHPTDILTSSTTIRMF  
MHGAAQSRVDSLVLDMLLPKQMILQLVKSI LERRLVLAGATGIGKSKLAKTLAAYVSIR  
TNQSEDSIVNISIPENNKEELLQVERRLEKILRSKESCIVILDNIPKNRIAFVVSVFANV  
PLQNNEGPFVVCTVNRYQIPELQIHNFKMSVMSNRLEGFILRYLRRRAVEDEYRLTVQM  
PSELFKIIDFFPIALQAVNNFIEKTNSVDVTVGPRACLNCP LTVDGSREWFIRLWNNFI  
PYLERVARDGKKNLRLSLHFLRGSHRHRL

*FIG. 4**21/99*

Length of tb3-m5.pro from cDNA pTB54 : 1528 aa; +1 at: 1;  
 Listed (Ordinary) from: 1 to: 1528; din, 23 apr 1996 11:49

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp	15
Ala Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg	30
Asp Ile Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln Leu	45
Ile Asn Val Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr	60
Lys Arg Leu Ala Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr	75
Cys Leu Asp Tyr Leu Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu	90
Thr Lys Thr Asp Ile Asp Ser Gly Asn Leu Gly Ala Val Leu Gln	105
Leu Leu Phe Leu Leu Ser Thr Tyr Lys Gln Lys Leu Arg Gln Leu	120
Lys Lys Asp Gln Lys Lys Leu Glu Gln Leu Pro Thr Ser Ile Met	135
Pro Pro Ala Val Ser Lys Leu Pro Ser Pro Arg Val Ala Thr Ser	150
Ala Thr Ala Ser Ala Thr Asn Pro Asn Ser Asn Phe Pro Gln Met	165
Ser Thr Ser Arg Leu Gln Thr Pro Gln Ser Arg Ile Ser Lys Ile	180
Asp Ser Ser Lys Ile Gly Ile Lys Pro Lys Thr Ser Gly Leu Lys	195
Pro Pro Ser Ser Ser Thr Thr Ser Ser Asn Asn Thr Asn Ser Phe	210
Arg Pro Ser Ser Arg Ser Ser Gly Asn Asn Asn Val Gly Ser Thr	225
Ile Ser Thr Ser Ala Lys Ser Leu Glu Ser Ser Ser Thr Tyr Ser	240
Ser Ile Ser Asn Leu Asn Arg Pro Thr Ser Gln Leu Gln Lys Pro	255
Ser Arg Pro Gln Thr Gln Leu Val Arg Val Ala Thr Thr Thr Lys	270
Ile Gly Ser Ser Lys Leu Ala Ala Pro Lys Ala Val Ser Thr Pro	285
Lys Leu Ala Ser Val Lys Thr Ile Gly Ala Lys Gln Glu Pro Asp	300
Asn Ser Gly Gly Gly Gly Gly Gly Met Leu Lys Leu Lys Leu Phe	315
Ser Ser Lys Asn Pro Ser Ser Ser Ser Asn Ser Pro Gln Pro Thr	330
Arg Lys Ala Ala Ala Val Pro Gln Gln Gln Thr Leu Ser Lys Ile	345
Ala Ala Pro Val Lys Ser Gly Leu Lys Pro Pro Thr Ser Lys Leu	360
Gly Ser Ala Thr Ser Met Ser Lys Leu Cys Thr Pro Lys Val Ser	375
Tyr Arg Lys Thr Asp Ala Pro Ile Ile Ser Gln Gln Asp Ser Lys	390

*FIG. 4 CONTINUED.**22/99*

Arg Cys Ser Lys Ser Ser Glu Glu Glu Ser Gly Tyr Ala Gly Phe	405
Asn Ser Thr Ser Pro Thr Ser Ser Ser Thr Glu Gly Ser Leu Ser	420
Met His Ser Thr Ser Ser Lys Ser Ser Thr Ser Asp Glu Lys Ser	435
Pro Ser Ser Asp Asp Leu Thr Leu Asn Ala Ser Ile Val Thr Ala	450
Ile Arg Gln Pro Ile Ala Ala Thr Pro Val Ser Pro Asn Ile Ile	465
Asn Lys Pro Val Glu Glu Lys Pro Thr Leu Ala Val Lys Gly Val	480
Lys Ser Thr Ala Lys Lys Asp Pro Pro Pro Ala Val Pro Pro Arg	495
Asp Thr Gln Pro Thr Ile Gly Val Val Ser Pro Ile Met Ala His	510
Lys Lys Leu Thr Asn Asp Pro Val Ile Ser Glu Lys Pro Glu Pro	525
Glu Lys Leu Gln Ser Met Ser Ile Asp Thr Thr Asp Val Pro Pro	540
Leu Pro Pro Leu Lys Ser Val Val Pro Leu Lys Met Thr Ser Ile	555
Arg Gln Pro Pro Thr Tyr Asp Val Leu Leu Lys Gln Gly Lys Ile	570
Thr Ser Pro Val Lys Ser Phe Gly Tyr Glu Gln Ser Ser Ala Ser	585
Glu Asp Ser Ile Val Ala His Ala Ser Ala Gln Val Thr Pro Pro	600
Thr Lys Thr Ser Gly Asn His Ser Leu Glu Arg Arg Met Gly Lys	615
Asn Lys Thr Ser Glu Ser Ser Gly Tyr Thr Ser Asp Ala Gly Val	630
Ala Met Cys Ala Lys Met Arg Glu Lys Leu Lys Glu Tyr Asp Asp	645
Met Thr Arg Arg Ala Gln Asn Gly Tyr Pro Asp Asn Phe Glu Asp	660
Ser Ser Ser Leu Ser Ser Gly Ile Ser Asp Asn Asn Glu Leu Asp	675
Asp Ile Ser Thr Asp Asp Leu Ser Gly Val Asp Met Ala Thr Val	690
Ala Ser Lys His Ser Asp Tyr Ser His Phe Val Arg His Pro Thr	705
Ser Ser Ser Ser Lys Pro Arg Val Pro Ser Arg Ser Ser Thr Ser	720
Val Asp Ser Arg Ser Arg Ala Glu Gln Glu Asn Val Tyr Lys Leu	735
Leu Ser Gln Cys Arg Thr Ser Gln Arg Gly Ala Ala Ala Thr Ser	750
Thr Phe Gly Gln His Ser Leu Arg Ser Pro Gly Tyr Ser Ser Tyr	765

*FIG. 4 CONTINUED.**23/99*

Ser Pro His Leu Ser Val Ser Ala Asp Lys Asp Thr Met Ser Met	780
His Ser Gln Thr Ser Arg Arg Pro Ser Ser Gln Lys Pro Ser Tyr	795
Ser Gly Gln Phe His Ser Leu Asp Arg Lys Cys His Leu Gln Glu	810
Phe Thr Ser Thr Glu His Arg Met Ala Ala Leu Leu Ser Pro Arg	825
Arg Val Pro Asn Ser Met Ser Lys Tyr Asp Ser Ser Gly Ser Tyr	840
Ser Ala Arg Ser Arg Gly Gly Ser Ser Thr Gly Ile Tyr Gly Glu	855
Thr Phe Gln Leu His Arg Leu Ser Asp Glu Lys Ser Pro Ala His	870
Ser Ala Lys Ser Glu Met Gly Ser Gln Leu Ser Leu Ala Ser Thr	885
Thr Ala Tyr Gly Ser Leu Asn Glu Lys Tyr Glu His Ala Ile Arg	900
Asp Met Ala Arg Asp Leu Glu Cys Tyr Lys Asn Thr Val Asp Ser	915
Leu Thr Lys Lys Gln Glu Asn Tyr Gly Ala Leu Phe Asp Leu Phe	930
Glu Gln Lys Leu Arg Lys Leu Thr Gln His Ile Asp Arg Ser Asn	945
Leu Lys Pro Glu Glu Ala Ile Arg Phe Arg Gln Asp Ile Ala His	960
Leu Arg Asp Ile Ser Asn His Leu Ala Ser Asn Ser Ala His Ala	975
Asn Glu Gly Ala Gly Glu Leu Leu Arg Gln Pro Ser Leu Glu Ser	990
Val Ala Ser His Arg Ser Ser Met Ser Ser Ser Ser Lys Ser Ser	1005
Lys Gln Glu Lys Ile Ser Leu Ser Ser Phe Gly Lys Asn Lys Lys	1020
Ser Trp Ile Arg Ser Ser Leu Ser Lys Phe Thr Lys Lys Lys Asn	1035
Lys Asn Tyr Asp Glu Ala His Met Pro Ser Ile Ser Gly Ser Gln	1050
Gly Thr Leu Asp Asn Ile Asp Val Ile Glu Leu Lys Gln Glu Leu	1065
Lys Glu Arg Asp Ser Ala Leu Tyr Glu Val Arg Leu Asp Asn Leu	1080
Asp Arg Ala Arg Glu Val Asp Val Leu Arg Glu Thr Val Asn Lys	1095
Leu Lys Thr Glu Asn Lys Gln Leu Lys Lys Glu Val Asp Lys Leu	1110
Thr Asn Gly Pro Ala Thr Arg Ala Ser Ser Arg Ala Ser Ile Pro	1125
Val Ile Tyr Asp Asp Glu His Val Tyr Asp Ala Ala Cys Ser Ser	1140

FIG. 4 CONTINUED.

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Thr Ser Ala Ser Gln Ser Ser Lys Arg Ser Ser Gly Cys Asn Ser	1155
Ile Lys Val Thr Val Asn Val Asp Ile Ala Gly Glu Ile Ser Ser	1170
Ile Val Asn Pro Asp Lys Glu Ile Ile Val Gly Tyr Leu Ala Met	1185
Ser Thr Ser Gln Ser Cys Trp Lys Asp Ile Asp Val Ser Ile Leu	1200
Gly Leu Phe Glu Val Tyr Leu Ser Arg Ile Asp Val Glu His Gln	1215
Leu Gly Ile Asp Ala Arg Asp Ser Ile Leu Gly Tyr Gln Ile Gly	1230
Glu Leu Arg Arg Val Ile Gly Asp Ser Thr Thr Met Ile Thr Ser	1245
His Pro Thr Asp Ile Leu Thr Ser Ser Thr Thr Ile Arg Met Phe	1260
Met His Gly Ala Ala Gln Ser Arg Val Asp Ser Leu Val Leu Asp	1275
Met Leu Leu Pro Lys Gln Met Ile Leu Gln Leu Val Lys Ser Ile	1290
Leu Thr Glu Arg Arg Leu Val Leu Ala Gly Ala Thr Gly Ile Gly	1305
Lys Ser Lys Leu Ala Lys Thr Leu Ala Ala Tyr Val Ser Ile Arg	1320
Thr Asn Gln Ser Glu Asp Ser Ile Val Asn Ile Ser Ile Pro Glu	1335
Asn Asn Lys Glu Glu Leu Leu Gln Val Glu Arg Arg Leu Glu Lys	1350
Ile Leu Arg Ser Lys Glu Ser Cys Ile Val Ile Leu Asp Asn Ile	1365
Pro Lys Asn Arg Ile Ala Phe Val Val Ser Val Phe Ala Asn Val	1380
Pro Leu Gln Asn Asn Glu Gly Pro Phe Val Val Cys Thr Val Asn	1395
Arg Tyr Gln Ile Pro Glu Leu Gln Ile His His Asn Phe Lys Met	1410
Ser Val Met Ser Asn Arg Leu Glu Gly Phe Ile Leu Arg Tyr Leu	1425
Arg Arg Arg Ala Val Glu Asp Glu Tyr Arg Leu Thr Val Gln Met	1440
Pro Ser Glu Leu Phe Lys Ile Ile Asp Phe Phe Pro Ile Ala Leu	1455
Gln Ala Val Asn Asn Phe Ile Glu Lys Thr Asn Ser Val Asp Val	1470
Thr Val Gly Pro Arg Ala Cys Leu Asn Cys Pro Leu Thr Val Asp	1485
Gly Ser Arg Glu Trp Phe Ile Arg Leu Trp Asn Glu Asn Phe Ile	1500
Pro Tyr Leu Glu Arg Val Ala Arg Asp Gly Lys Lys Asn Leu Arg	1515
Ser Leu His Phe Leu Arg Gly Ser His Arg His Arg Leu	



FIG. 5.

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Annotated sequence of 7A variant of UNC-53

10 20 30 40 50 60  
MTTSNVELIP IYTDWANRHL SKGSLSKSIR DISNDFRDYR LVSOLINVIV PINEFSPAFT  
 start tb6 and tb3 similarity to amino-termini of alfa-actinin,

70 80 90 100 110 120  
KRLAKITSNL DGLETCLDYL KNLGLDCSKL TKTDIDSGNL GAVLOLLFLL STYKOKLRQL  
 beta-spectrin, dystrophin, fimbrin, filamin actin-binding site 1  
 (114 - 133)

130 140 150 160 170 180  
KKDOKKLEOL PTSIMPPAVS KLPSRPVATS ATASATNPNS NFPQMSTSRRL QTPQSRISKI  
 Start S4 poss. start tblb & tb6 & tbl lamda clone

190 200 210 220 230 240  
 DSSKIGIKPK TSGLKPPSSS TTSSNNTNSF RPSSRSSGNN NVGSTISTSA KSLESSSTYS

250 260 270 280 290 300  
 SISNLNRPTS QLQKPSRPQT QLVRVATTTK IGSSKLAAPK AVSTPKLASV KTIGAKQEPD

310 320 330 340 350 360  
 NSGGGGGGML KLKLFSSKNP SSSSNSPQPT RKAAPVQQQ TSKIAAPVK SGLKPPTSKL

370 380 390 400 410 420  
 GSATSMKLC TPKVSYRKTD APIISQODSK RCSKSSEES GYAGFNSTSP TSSSTEGSLS

430 440 450 460 470 480  
MHSTSSKSST SDEKSPSSDD LTLNASIVTA IRQPIAATPV SPNIINKPVE EKPTLAVKGV  
 poss. start tb22

490 500 510 520 530 540  
KSTAKKDPPP AVPPRDTQPT IGVVSPIMAH KKLTNDPVIS EKPEPEKLQS MSIDTTDVPP  
 SH3-binding 1 SH3-

550 560 570 580 590 600  
LPPLKSVVPL KMTSIRQPPT YDVLLKQGKI TSPVKSFGEY QSSASEDSIV AHASQVTPP  
 binding 2

610 620 630 640 650 660  
 TKTSGNHSLE RRMGKNKTSE SSGYTS DAGV AMCAKMREKL KEYDDMTRRA QNGYPDNFED

670 680 690 700 710 720  
 SSSLSSGISD NNELDDISTD DLSGVDMATV ASKHSDYSHF VRHPTSSSSK PRVPSRSSTS

730 740 750 760 770 780  
 VDSRSRAEQE NVYKLLSQCR TSQRGAAATS TFGQHS LRSP GYSSYSPLHS VSADKDTMSM

790 800 810 820 830 840  
HSQTSRRPSS QKPSYSGOFH SLDRKCHLOE FTSTEHRMAA LLSPRRVPNS MSKYDSSGSY  
 Kohara Exon deleted in cDNA YK25D6

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FIG. 5 CONTINUED.

10	20	30	40	50	60	70
<u>AALNASGMSR SMILLESLSPPPPRRHOSPA DSCIITASPS APRRSHSPRG PTARIPLSLA SSPVHVNNNW</u> predicted exon (alternative/additional to Kohara exon to be inserted after aminoacid 838 )						
850	860	870	880	890	900	
SARSRGGSST	GIYGETFQLH	RLSDEKSPAH	SAKSEMGSQ	SLASTTAYGS	LNEKYEHAI	R
910	920	930	940	950	960	
DMARDLECYK	NTVDSLTKKQ	ENYGALFDLF	EQKLRKLTQH	IDRSNLKPEE	AIRFRQDIAH	
970	980	990	1000	1010	1020	
LRDISNHLAS	NSAHANEGAG	ELLRQPSLES	VASHRSSMSS	SSKSSKQEKI	SLSSFGKNNK	
1030	1040	1050	1060	1070	1080	
SWIRSSLSKF	TKKKKNKYDE	AHMPISGSQ	GTLDNIDVIE	LKQELKERDS	ALYEVRLDNL	
candidate nuclear Start GP45 localization signal						
1090	1100	1110	1120	1130	1140	
DRAREVDVLR	ETVNKLKTEN	KOLKKEVDKL	TNGPATRASS	RASIPVIYDD	EHVYDAACSS	
actin binding site 2						
(1097-1116)						
* * *						
candidate leucine zipper.pattern						
1150	1160	1170	1180	1190	1200	
TSASQSSKRS	SGCNSIKVTV	NVDIAGEISS	IVNPDKEIIV	GYLAMSTSQS	CWKDIDVSIL	
1210	1220	1230	1240	1250	1260	
GLFEVYLSRI	DVEHQLGIDA	RDSILGYQIG	ELRRVIGDST	TMITSHPTDI	LTSSTTIRMF	
1270	1280	1290	1300	1310	1320	
MHGAAQSRVD	SLVLDMLLPK	QMILQLVKSI	LTERRLVLAG	ATGIGKSKLA	KTAAAYVSIR	
* * * nucleotide binding pocket						
candidate leucine zipper.pattern						
1330	1340	1350	1360	1370	1380	
TNQSEDSIVN	ISIPENNKEE	LLQVERRLEK	ILRSKESCIV	ILDNIPKNRI	AFVVSVFANV	
1390	1400	1410	1420	1430	1440	
PLQNNEGPFV	VCTVNRYP	ELQIHNFMM	SVMSNRLEGF	ILRYLRRRAV	EDEYRLTVQM	
1450	1460	1470	1480	1490	1500	
PSELFKIIDF	FPIALQAVNN	FIEKTNSVDV	TVGPRACLNC	PLTVDGSREW	FIRLWNNFI	
end GP45						
1510	1520	1530	1540	1550	1560	
PYLERVARDG	KKTFGRCTSF	EDPTDIVSEK	WPWFDGENPE	NVLKRLQLQD	LVPSPANSSR	
1570	1580					
QHFNPLESLI	QLHATKHQTI	DNI				

FIG. 6.

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Length of Untitled : 1583 aa from cDNA pTB72; +1 at: 1;  
 Listed (Ordinary) from: 1 to: 1583; din, 23 apr 1996 11:37

Met Thr Thr Ser Asn Val Glu Leu Ile Pro Ile Tyr Thr Asp Trp	15
Ala Asn Arg His Leu Ser Lys Gly Ser Leu Ser Lys Ser Ile Arg	30
Asp Ile Ser Asn Asp Phe Arg Asp Tyr Arg Leu Val Ser Gln Leu	45
Ile Asn Val Ile Val Pro Ile Asn Glu Phe Ser Pro Ala Phe Thr	60
Lys Arg Leu Ala Lys Ile Thr Ser Asn Leu Asp Gly Leu Glu Thr	75
Cys Leu Asp Tyr Leu Lys Asn Leu Gly Leu Asp Cys Ser Lys Leu	90
Thr Lys Thr Asp Ile Asp Ser Gly Asn Leu Gly Ala Val Leu Gln	105
Leu Leu Phe Leu Leu Ser Thr Tyr Lys Gln Lys Leu Arg Gln Leu	120
Lys Lys Asp Gln Lys Lys Leu Glu Gln Leu Pro Thr Ser Ile Met	135
Pro Pro Ala Val Ser Lys Leu Pro Ser Pro Arg Val Ala Thr Ser	150
Ala Thr Ala Ser Ala Thr Asn Pro Asn Ser Asn Phe Pro Gln Met	165
Ser Thr Ser Arg Leu Gln Thr Pro Gln Ser Arg Ile Ser Lys Ile	180
Asp Ser Ser Lys Ile Gly Ile Lys Pro Lys Thr Ser Gly Leu Lys	195
Pro Pro Ser Ser Ser Thr Thr Ser Ser Asn Asn Thr Asn Ser Phe	210
Arg Pro Ser Ser Arg Ser Ser Gly Asn Asn Asn Val Gly Ser Thr	225
Ile Ser Thr Ser Ala Lys Ser Leu Glu Ser Ser Ser Thr Tyr Ser	240
Ser Ile Ser Asn Leu Asn Arg Pro Thr Ser Gln Leu Gln Lys Pro	255
Ser Arg Pro Gln Thr Gln Leu Val Arg Val Ala Thr Thr Thr Lys	270
Ile Gly Ser Ser Lys Leu Ala Ala Pro Lys Ala Val Ser Thr Pro	285
Lys Leu Ala Ser Val Lys Thr Ile Gly Ala Lys Gln Glu Pro Asp	300
Asn Ser Gly Gly Gly Gly Gly Gly Met Leu Lys Leu Lys Leu Phe	315
Ser Ser Lys Asn Pro Ser Ser Ser Ser Asn Ser Pro Gln Pro Thr	330
Arg Lys Ala Ala Ala Val Pro Gln Gln Gln Thr Leu Ser Lys Ile	345
Ala Ala Pro Val Lys Ser Gly Leu Lys Pro Pro Thr Ser Lys Leu	360
Gly Ser Ala Thr Ser Met Ser Lys Leu Cys Thr Pro Lys Val Ser	375

FIG. 6 CONTINUED.

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Tyr Arg Lys Thr Asp Ala Pro Ile Ile Ser Gln Gln Asp Ser Lys	390
Arg Cys Ser Lys Ser Ser Glu Glu Glu Ser Gly Tyr Ala Gly Phe	405
Asn Ser Thr Ser Pro Thr Ser Ser Ser Thr Glu Gly Ser Leu Ser	420
Met His Ser Thr Ser Ser Lys Ser Ser Thr Ser Asp Glu Lys Ser	435
Pro Ser Ser Asp Asp Leu Thr Leu Asn Ala Ser Ile Val Thr Ala	450
Ile Arg Gln Pro Ile Ala Ala Thr Pro Val Ser Pro Asn Ile Ile	465
Asn Lys Pro Val Glu Glu Lys Pro Thr Leu Ala Val Lys Gly Val	480
Lys Ser Thr Ala Lys Lys Asp Pro Pro Pro Ala Val Pro Pro Arg	495
Asp Thr Gln Pro Thr Ile Gly Val Val Ser Pro Ile Met Ala His	510
Lys Lys Leu Thr Asn Asp Pro Val Ile Ser Glu Lys Pro Glu Pro	525
Glu Lys Leu Gln Ser Met Ser Ile Asp Thr Thr Asp Val Pro Pro	540
Leu Pro Pro Leu Lys Ser Val Val Pro Leu Lys Met Thr Ser Ile	555
Arg Gln Pro Pro Thr Tyr Asp Val Leu Leu Lys Gln Gly Lys Ile	570
Thr Ser Pro Val Lys Ser Phe Gly Tyr Glu Gln Ser Ser Ala Ser	585
Glu Asp Ser Ile Val Ala His Ala Ser Ala Gln Val Thr Pro Pro	600
Thr Lys Thr Ser Gly Asn His Ser Leu Glu Arg Arg Met Gly Lys	615
Asn Lys Thr Ser Glu Ser Ser Gly Tyr Thr Ser Asp Ala Gly Val	630
Ala Met Cys Ala Lys Met Arg Glu Lys Leu Lys Glu Tyr Asp Asp	645
Met Thr Arg Arg Ala Gln Asn Gly Tyr Pro Asp Asn Phe Glu Asp	660
Ser Ser Ser Leu Ser Ser Gly Ile Ser Asp Asn Asn Glu Leu Asp	675
Asp Ile Ser Thr Asp Asp Leu Ser Gly Val Asp Met Ala Thr Val	690
Ala Ser Lys His Ser Asp Tyr Ser His Phe Val Arg His Pro Thr	705
Ser Ser Ser Ser Lys Pro Arg Val Pro Ser Arg Ser Ser Thr Ser	720
Val Asp Ser Arg Ser Arg Ala Glu Gln Glu Asn Val Tyr Lys Leu	735
Leu Ser Gln Cys Arg Thr Ser Gln Arg Gly Ala Ala Ala Thr Ser	750
Thr Phe Gly Gln His Ser Leu Arg Ser Pro Gly Tyr Ser Ser Tyr	765
Ser Pro His Leu Ser Val Ser Ala Asp Lys Asp Thr Met Ser Met	780

*FIG. 6 CONTINUED.**29/99*

His Ser Gln Thr Ser Arg Arg Pro Ser Ser Gln Lys Pro Ser Tyr	795
Ser Gly Gln Phe His Ser Leu Asp Arg Lys Cys His Leu Gln Glu	810
Phe Thr Ser Thr Glu His Arg Met Ala Ala Leu Leu Ser Pro Arg	825
Arg Val Pro Asn Ser Met Ser Lys Tyr Asp Ser Ser Gly Ser Tyr	840
Ser Ala Arg Ser Arg Gly Gly Ser Ser Thr Gly Ile Tyr Gly Glu	855
Thr Phe Gln Leu His Arg Leu Ser Asp Glu Lys Ser Pro Ala His	870
Ser Ala Lys Ser Glu Met Gly Ser Gln Leu Ser Leu Ala Ser Thr	885
Thr Ala Tyr Gly Ser Leu Asn Glu Lys Tyr Glu His Ala Ile Arg	900
Asp Met Ala Arg Asp Leu Glu Cys Tyr Lys Asn Thr Val Asp Ser	915
Leu Thr Lys Lys Gln Glu Asn Tyr Gly Ala Leu Phe Asp Leu Phe	930
Glu Gln Lys Leu Arg Lys Leu Thr Gln His Ile Asp Arg Ser Asn	945
Leu Lys Pro Glu Glu Ala Ile Arg Phe Arg Gln Asp Ile Ala His	960
Leu Arg Asp Ile Ser Asn His Leu Ala Ser Asn Ser Ala His Ala	975
Asn Glu Gly Ala Gly Glu Leu Leu Arg Gln Pro Ser Leu Glu Ser	990
Val Ala Ser His Arg Ser Ser Met Ser Ser Ser Ser Lys Ser Ser	1005
Lys Gln Glu Lys Ile Ser Leu Ser Ser Phe Gly Lys Asn Lys Lys	1020
Ser Trp Ile Arg Ser Ser Leu Ser Lys Phe Thr Lys Lys Lys Asn	1035
Lys Asn Tyr Asp Glu Ala His Met Pro Ser Ile Ser Gly Ser Gln	1050
Gly Thr Leu Asp Asn Ile Asp Val Ile Glu Leu Lys Gln Glu Leu	1065
Lys Glu Arg Asp Ser Ala Leu Tyr Glu Val Arg Leu Asp Asn Leu	1080
Asp Arg Ala Arg Glu Val Asp Val Leu Arg Glu Thr Val Asn Lys	1095
Leu Lys Thr Glu Asn Lys Gln Leu Lys Lys Glu Val Asp Lys Leu	1110
Thr Asn Gly Pro Ala Thr Arg Ala Ser Ser Arg Ala Ser Ile Pro	1125
Val Ile Tyr Asp Asp Glu His Val Tyr Asp Ala Ala Cys Ser Ser	1140
Thr Ser Ala Ser Gln Ser Ser Lys Arg Ser Ser Gly Cys Asn Ser	1155,
Ile Lys Val Thr Val Asn Val Asp Ile Ala Gly Glu Ile Ser Ser	1170
Ile Val Asn Pro Asp Lys Glu Ile Ile Val Gly Tyr Leu Ala Met	1185

*FIG. 6 CONTINUED.**30/99*

Ser Thr Ser Gln Ser Cys Trp Lys Asp Ile Asp Val Ser Ile Leu	1200
Gly Leu Phe Glu Val Tyr Leu Ser Arg Ile Asp Val Glu His Gln	1215
Leu Gly Ile Asp Ala Arg Asp Ser Ile Leu Gly Tyr Gln Ile Gly	1230
Glu Leu Arg Arg Val Ile Gly Asp Ser Thr Thr Met Ile Thr Ser	1245
His Pro Thr Asp Ile Leu Thr Ser Ser Thr Thr Ile Arg Met Phe	1260
Met His Gly Ala Ala Gln Ser Arg Val Asp Ser Leu Val Leu Asp	1275
Met Leu Leu Pro Lys Gln Met Ile Leu Gln Leu Val Lys Ser Ile	1290
Leu Thr Glu Arg Arg Leu Val Leu Ala Gly Ala Thr Gly Ile Gly	1305
Lys Ser Lys Leu Ala Lys Thr Leu Ala Ala Tyr Val Ser Ile Arg	1320
Thr Asn Gln Ser Glu Asp Ser Ile Val Asn Ile Ser Ile Pro Glu	1335
Asn Asn Lys Glu Glu Leu Leu Gln Val Glu Arg Arg Leu Glu Lys	1350
Ile Leu Arg Ser Lys Glu Ser Cys Ile Val Ile Leu Asp Asn Ile	1365
Pro Lys Asn Arg Ile Ala Phe Val Val Ser Val Phe Ala Asn Val	1380
Pro Leu Gln Asn Asn Glu Gly Pro Phe Val Val Cys Thr Val Asn	1395
Arg Tyr Gln Ile Pro Glu Leu Gln Ile His His Asn Phe Lys Met	1410
Ser Val Met Ser Asn Arg Leu Glu Gly Phe Ile Leu Arg Tyr Leu	1425
Arg Arg Arg Ala Val Glu Asp Glu Tyr Arg Leu Thr Val Gln Met	1440
Pro Ser Glu Leu Phe Lys Ile Ile Asp Phe Phe Pro Ile Ala Leu	1455
Gln Ala Val Asn Asn Phe Ile Glu Lys Thr Asn Ser Val Asp Val	1470
Thr Val Gly Pro Arg Ala Cys Leu Asn Cys Pro Leu Thr Val Asp	1485
Gly Ser Arg Glu Trp Phe Ile Arg Leu Trp Asn Glu Asn Phe Ile	1500
Pro Tyr Leu Glu Arg Val Ala Arg Asp Gly Lys Lys Thr Phe Gly	1515
Arg Cys Thr Ser Phe Glu Asp Pro Thr Asp Ile Val Ser Lys Lys	1530
Trp Pro Trp Phe Asp Gly Glu Asn Pro Glu Asn Val Leu Lys Arg	1545
Leu Gln Leu Gln Asp Leu Val Pro Ser Pro Ala Asn Ser Ser Arg	1560
Gln His Phe Asn Pro Leu Glu Ser Leu Ile Gln Leu His Ala Thr	1575
Lys His Gln Thr Ile Asp Asn Ile	

FIG. 7.

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MTTSNVELIPIYTDWANRHLSKGSLSKSIIRDISNDFRDYRLVSQLINVIVPINEFSPAFT  
 KRLAKITSNLDGLETCLDYLNKLGDCSKLTKTIDIDSGNLGAVLQLLFLLSTYXXXXXXX  
 XXXXXXXXXXXXPTSIMPPAVSKLXXXXXXXXXXXXXXXXXXXXXFPQMSTSRLOTPOXXXXXX  
 XXXXXXXXXXXXTSGLKPXX  
 XXXNLNRPTSQLOKPSRPQTQLVRVATTTKIGSSKLAAPKAVSTPKLASVKTIGAKQEPD  
 NSXXXXXXXXXXXXXXXXXXXXXXXXXXXXQPTRKAAAVPQQOTLSKIAAPVKSGLKPPPTSKL  
 GSATSMKSLCTPKVSYRKTDAPIIISQODSKRCSKXXXXXXXXGYAGFNXXXXXXXXXXXXXX  
 XXXXXXXXXXXXXXXXXXXXDDLTLNASIVTAIRQPIAATPVSPNIINKPVEEKPTLAVKGV  
 KSTAKKDPFPAVPPRDTQPTIGVVSPIMAHKKLTNDPVISEKPEPEKLQSMSIDTTDXXX  
 XXXXXXXXXXXXMTSIRQPPTYDVLLKQKGKITSVPKSFGEQSSASEDSIVAHASAQVTPP  
 TKTSGNHSLERRMGKNKTSESSGYTSDAGVAMCAKMREKLKEYDDMTRRAQNGYPDNFED  
 XXXXXXXXXXXXNNELDDISTDDLSGVDMATVASKHSDYSHFVRHPXXXXXXXXXXXXXXXX  
 XXXXXXAEQENVYKLLSQCRTSQRGAATSTFGQHSLSRSPGYSSYSPHLSVSADKDTMSM  
 HSQTSRRPSSQKPSYSGQFHSLSDRKCHLQEFSTEHRMAALLSPRRVPNXXXXXXXXXXXX  
 XXXXXXXXXXXXIYGETFQLHRLSDEKSPAHSKSEMGSQSLASTTAYGSLNEKEYEHAIR  
 DMARDLECYKNTVDSLTKKQENYGALFDLFEQKLRLKTQHIDRSNLKPEEAIRFRQDIAH  
 LRDISNHLASNSAHANEGAGELLRQPSLEXXXXXXXXXXXXXXXXXXXXXXXXXXFGKNKK  
 SWIRSSLSKFTKKKNKNYDEAHMPSISGSQGTLDNIDVIELKQELKERDSALYEVRLDNL  
 DRAREVDVLRETVNKLKTENKQLKKEVDKLTNGPATRASSRASIPVIYDDEHVYDXXXXX  
 XXXXXXXXXXXXGCNXXXXXXXXXXXXXXXXXXXXXXXXXDEKIIIVGYLAMSTSQSCWKDIDVIL  
 GLFEVYLSRIDVEHQLGIDARDSILGYQIGELRRVIGDSTMITSHPTDILTSSTTIRMF  
 MHGAAQSRVDSLVLDMLLPKQMILQLVKSILTERRVLGATGIGKSKLAKTLAAVVSIR  
 TNQSEDSIVNISIPENNKEELLQVERRLEKILRSKESCIVILDNIPKNRIAFVSVFANV  
 PLQNNEGPFVCTVNRYQIPELQIHNFKMSVMSNRLEGFILRYLRRRAVEYRLTVQM  
 PSELFKIIDFFPIALQAVNNFIEKTNFVDVTVGPRACLNCPLTVDGSREWFIRLWNNFI  
 PYLERVARDGKKNLRLSLHFLRGSHRHRL

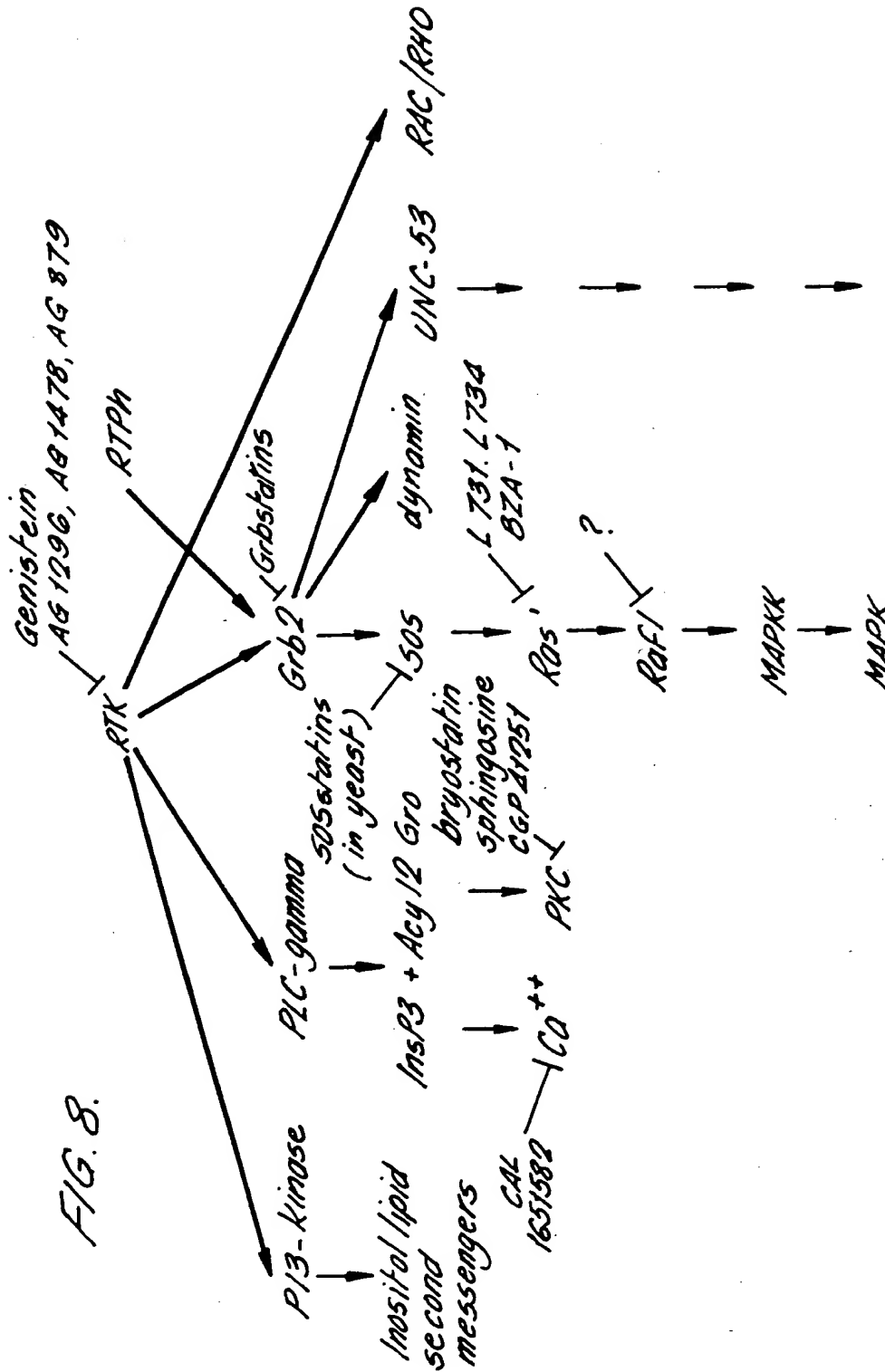
MTTSNVELIPIYTDWANRHLSKGSLSKSIIRDISNDFRDYRLVSQLINVIVPINEFSPAFT  
 KRLAKITSNLDGLETCLDYLNKLGDCSKLTKTIDIDSGNLGAVLQLLFLLSTYKOKLROL  
 KKDQKKLEQLPTSIMPPAVSKLPSPRVATSATASATNPNSNFPQMSTSRLOTPOSRSKI  
 DSSKIGIKPKTSGLKPPSSSTTSSNNTNSFRPSSRSSGNNNVGSTISTSAKSLESSSTYS  
 SLSNLNRPTSQLOKPSRPQTQLVRVATTTKIGSSKLAAPKAVSTPKLASVKTIGAKQEPD  
 NSGGGGGGMKLKLFSSKNPSSSSNSPQPTRKAAAVPQQOTLSKIAAPVKSGLKPPPTSKL  
 GSATSMKSLCTPKVSYRKTDAPIIISQODSKRCSKSSEEEESGYAGFNSTSPSSSTEGSLS  
 MHSTSSKSSTSDEKSPSSDDLTLNASIVTAIRQPIAATPVSPNIINKPVEEKPTLAVKGV  
 KSTAKKDPFPAVPPRDTQPTIGVVSPIMAHKKLTNDPVISEKPEPEKLQSMSIDTTDVPP  
 LPPLKSVVPLKMTSIRQPPTYDVLLKQKGKITSVPKSFGEQSSASEDSIVAHASAQVTPP  
 TKTSGNHSLERRMGKNKTSESSGYTSDAGVAMCAKMREKLKEYDDMTRRAQNGYPDNFED  
 SSSLSSGISDNNELDDISTDDLSGVDMATVASKHSDYSHFVRHPTSSSSKPRVPSRSSTS  
 VDSRSRAEQENVYKLLSQCRTSQRGAATSTFGQHSLSRSPGYSSYSPHLSVSADKDTMSM  
 HSQTSRRPSSQKPSYSGQFHSLSDRKCHLQEFSTEHRMAALLSPRRVPNSMSKYDSSGSY  
 SARSRGGSSTGIYGETFQLHRLSDEKSPAHSKSEMGSQSLASTTAYGSLNEKEYEHAIR  
 DMARDLECYKNTVDSLTKKQENYGALFDLFEQKLRLKTQHIDRSNLKPEEAIRFRQDIAH  
 LRDISNHLASNSAHANEGAGELLRQPSLESVASHRSSMSSSSKSSKOEKISLSSFGKNKK  
 SWIRSSLSKFTKKKNKNYDEAHMPSISGSQGTLDNIDVIELKQELKERDSALYEVRLDNL  
 DRAREVDVLRETVNKLKTENKQLKKEVDKLTNGPATRASSRASIPVIYDDEHVYDAACSS

*32/99**FIG. 7 CONTINUED.*

TSASOSSKRSSGCNSIKVTVNVDIAGEISSIVNPDKEIIVGYLAMSTSQSCWKDIDVSIL  
GLFEVYLSRIDVEHQLGIDARDSILGYQIGELRRVIGDSTTMITSHPTDILTSSTTIRMF  
MHGAAQSRVDSLVLDMLLPKQMILQLVKSI LERRVLGATGIGKSKLAKTLAAYVSIR  
TNQSEDSIVNISIPENNKEELLQVERRLEKILRSKESCIVILDNIPKNRIAFVVSVFANV  
PLQNNEGPFVVCTVNRVYQIPELQIHHNFKMSVMSNRLEGFILRYLRRRAVEDEYRLTVQM  
PSELFKIIDFFPIALQAVNNFIEKTSVDVTVGPRACLNCPLTVDGSREWFIRLWNEFI  
PYLERVARDGKKNLRLSLHFLRGSHRHRL



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FIG. 9.

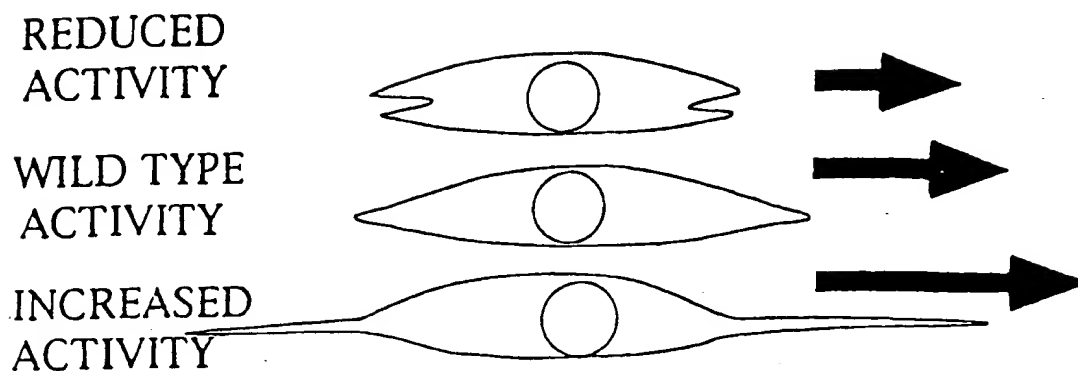


FIG. 10.

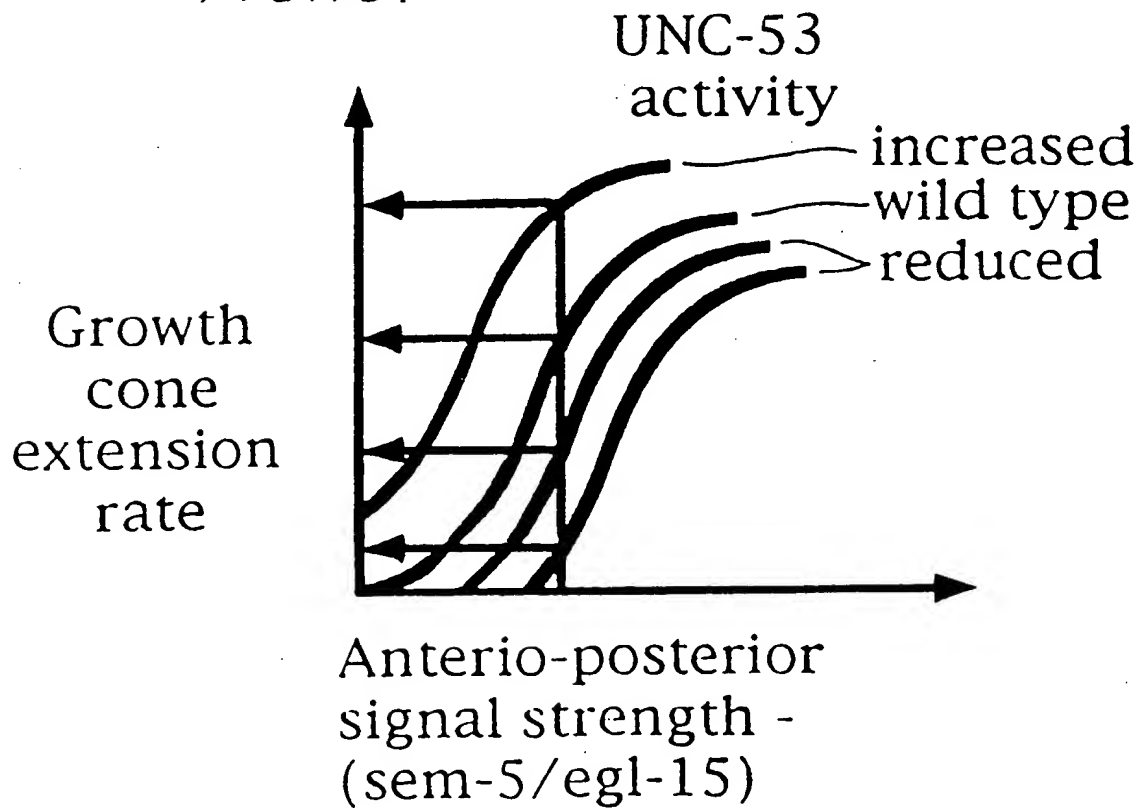
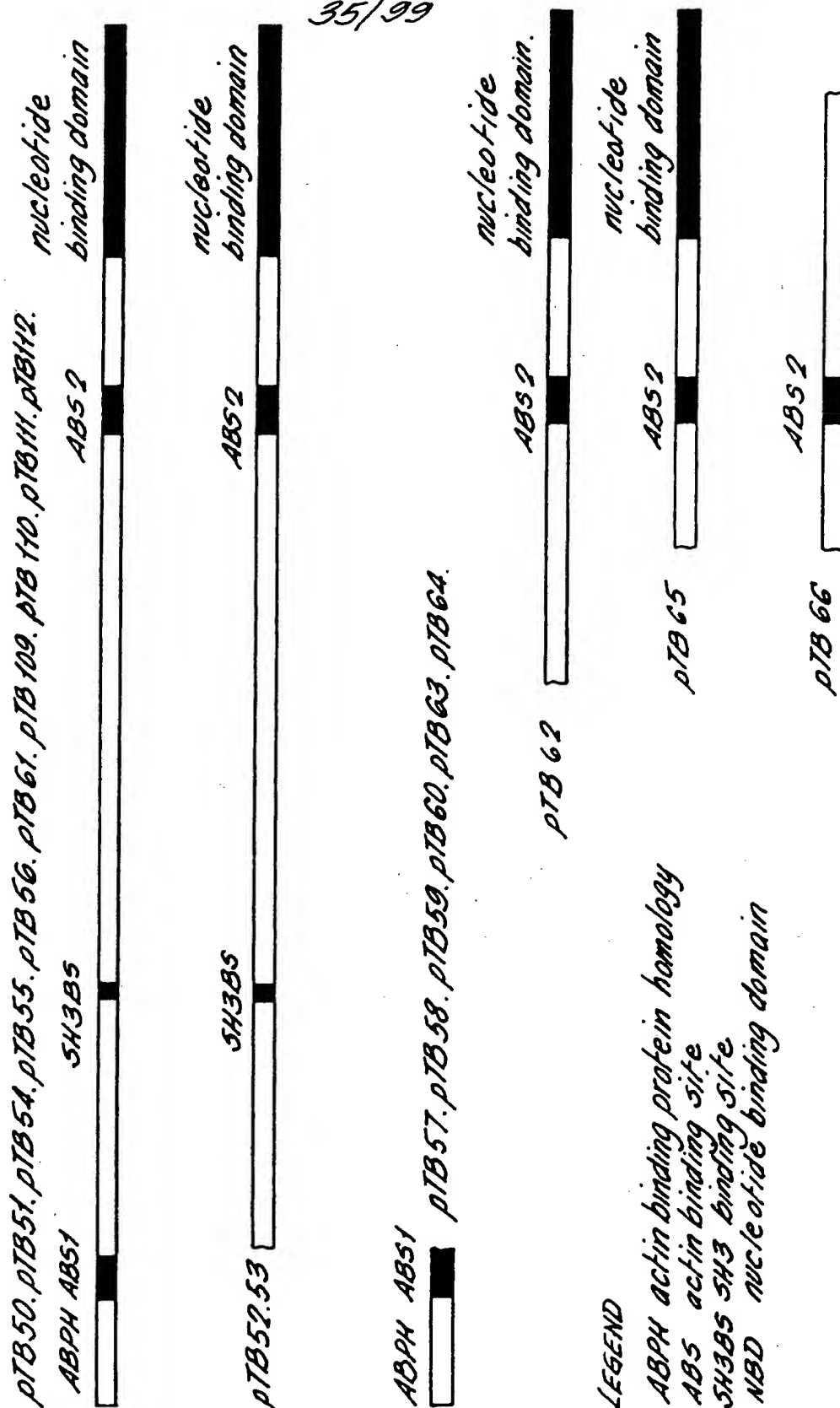


FIG. 11.



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FIG. 12.

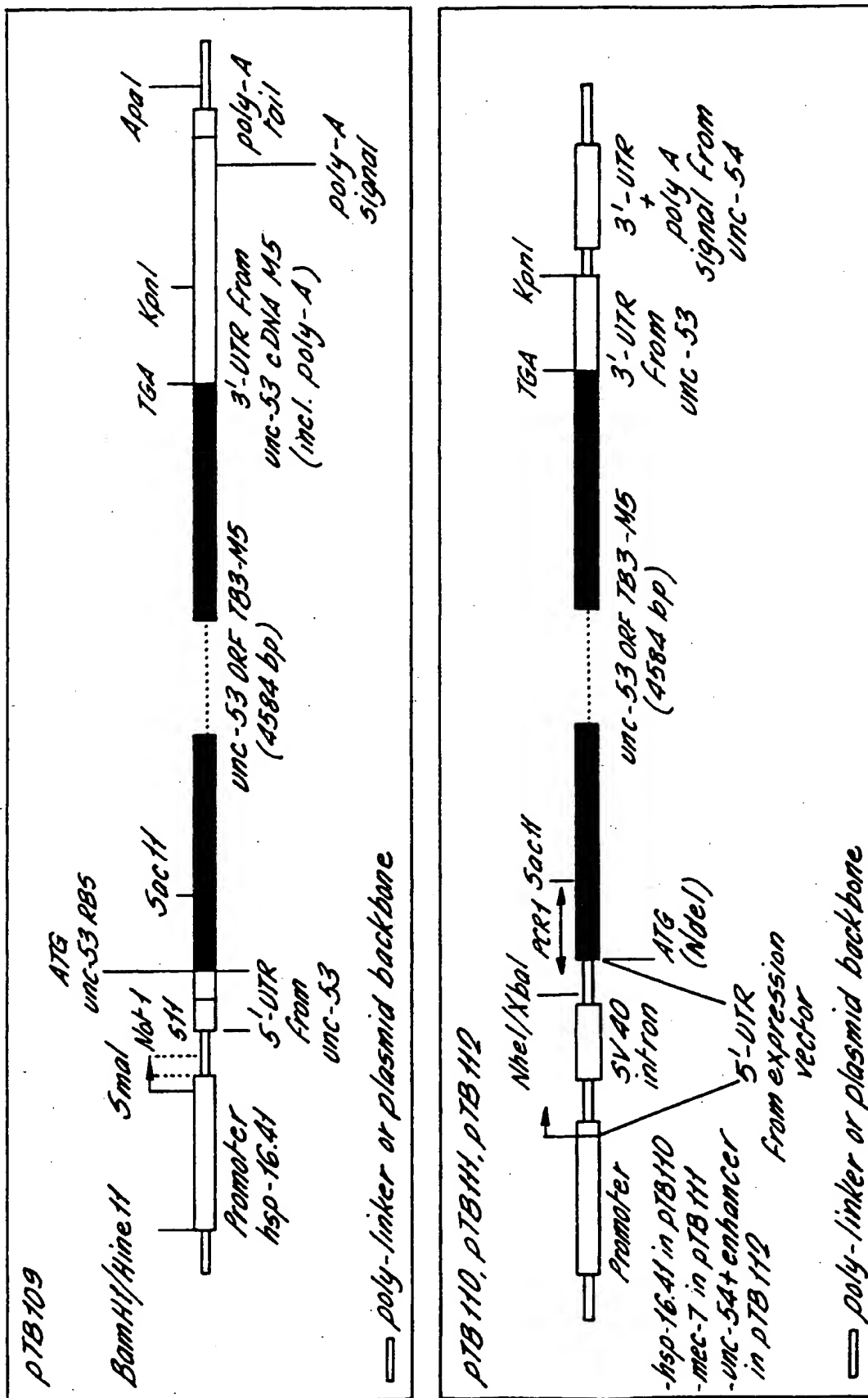
5' ataaqaatgcggccgcgccatgacgacgtcaaatgtagaattgata (oligo BG03)

5' ggaattccaaccatATGACGACGTCAAAATGTAGAATTGATA (oligo BG01)

ATGACGACGTCAAAATGTAGAATTGATACCAATCTACACGGATTGGCCCAATCGGCACCTTTCCG  
AAGGGCAGCTTATCAAAGTCGATTAGGGATATTCCCAATGATTTTTCGCGACTATCGACTGGTT  
TCTCAGCTTATTAAATGTGATCGTTCCGATCAACGAATTTCTCGCTGCATTTACGAAACGTTTG  
GCAGAAATCAGATCGAACCTGGATGGCCCTCGAAACGTGTCTCGACTACCTGAGAAATCTGGGT  
CTCGACTGCTCGAACTCACCAAAACCGATATCGACAGCGGAACTTGGGTGCAGTTCTCCAG  
CTGCTCTTCTGCTCTCCACCTACAGCAGAAAGCTTCGGCNACTGAGAAAGATCAGAGAGAA  
TTGGAGCAACTACCCACATCCATTATGCCACCCGGGTTTCTAAATTACCTCGCCACGCTGC  
(oligo BG02) GTAGGTAAATACGTTGGTGGCCCAACTcctagggc-5'

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FIG. 13.



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FIG. 14a.

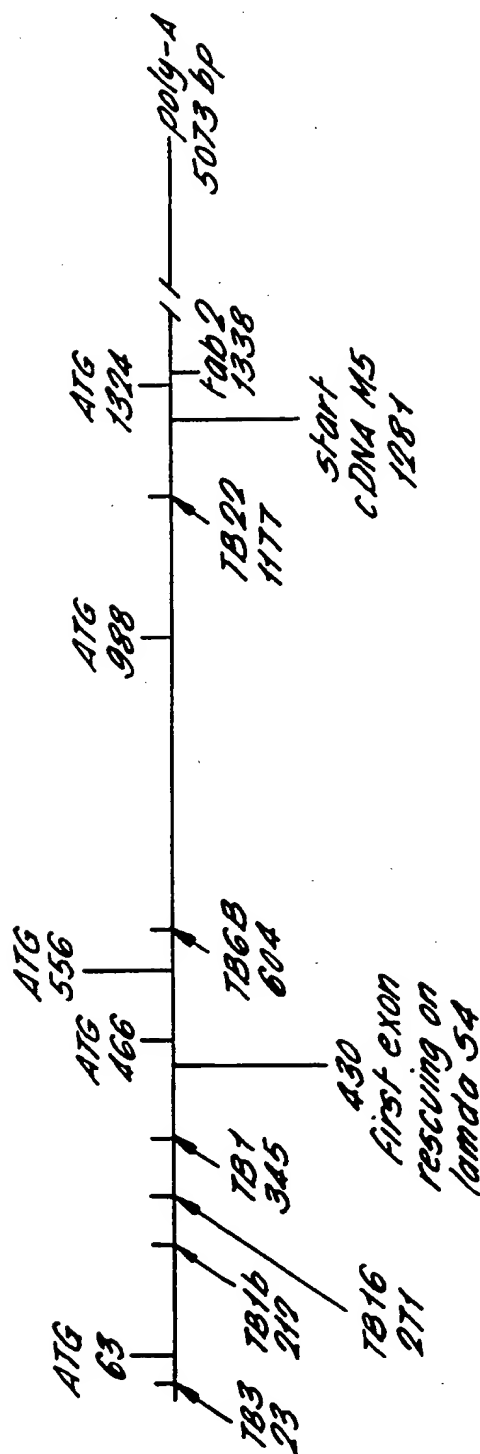
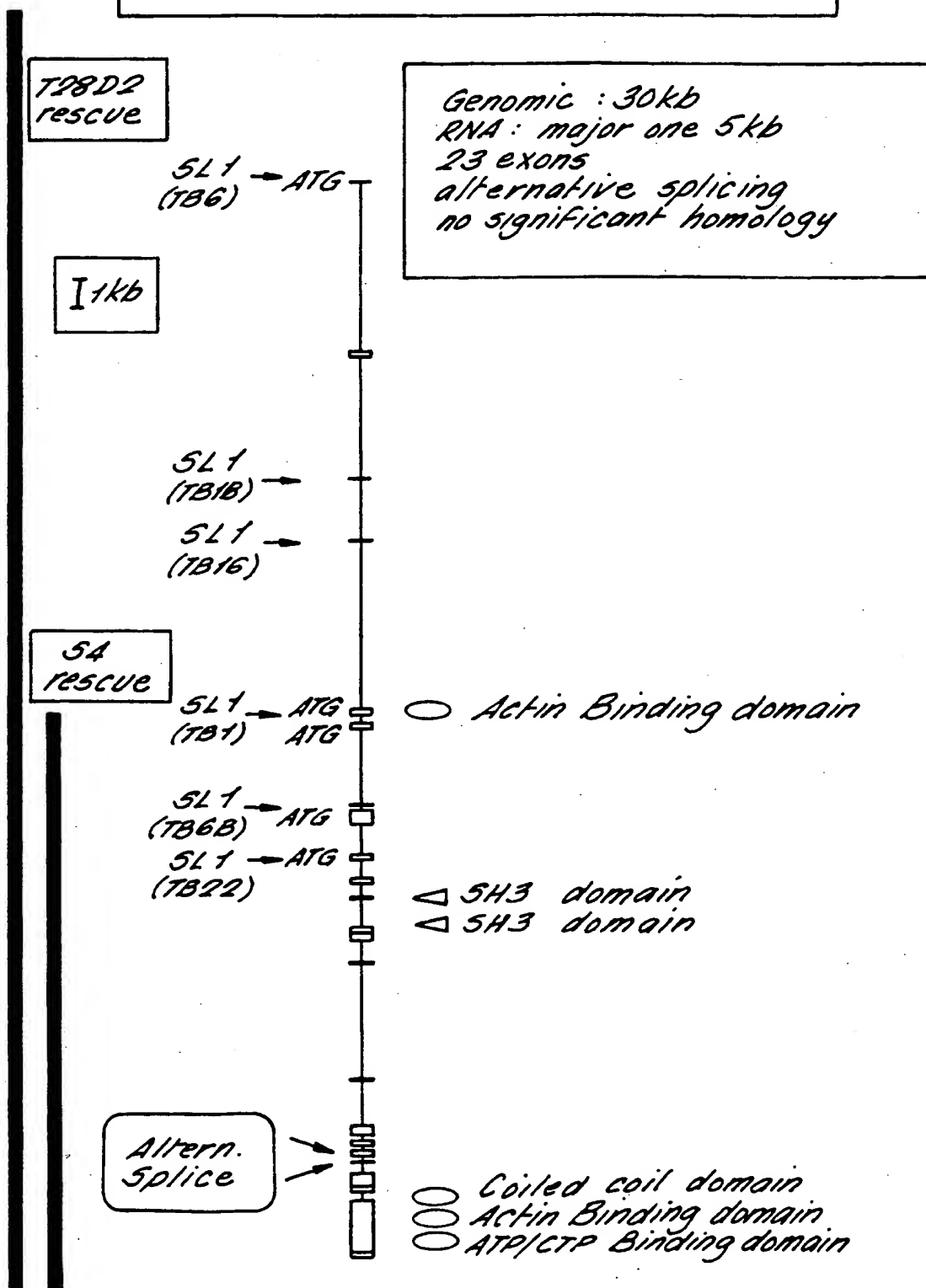


FIG. 14b. 39/99

## MOLECULAR DATA ON UNC-53



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FIG. 14c.

S4

5'  
gatcagaagaaattggagcaactacccacatccattatgccacccgcggtttctaagtgagt  
ttaattttgagtttacgactacaaaaatgtgttcttta

.....

ccgccttctgacttcgtgacgacagtctcgacacgtggggttgccaggtaggagtggatgagtc  
cgaaactgataagatagtcatttgagatc 3'

Co-ordinates in ACEDB.

5' begins at position 2260 in C09H10.

3' finishes at 3287 in F45 E10.

Total 16818 bp.





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FIG. 16.

LLFLLSTYKQKLRQLKKDQKKLEQLPTS unc-53 106 to 133  
 : | :|||: || |::  
 ETVNVNKLKTENKQLKKEVDKLTNGPAT unc-53 1093 to 1120

FIG. 17.

side on helix 1 4 7

XphPpxP

(a)	UNC-53	KK <u>D</u> PPPAVPPRDT
(b)	UNC-53	TTDVPPLPPLKS
(c)	mSOS	EVPVPPPVPPRR
(d)	mSOS	HLDSPPAIPPR
(e)	mSOS	HSIAGPPVPPR
(f)	SOS 1359	YRAVPPPLPPRRK
(g)	SOS 1377	GELSPPPIPPRLN
(h)	Dynammin	APAVPPARPGS
(i)	dynammin	PAVPPARP
(j)	PI3K p85	PPRPLPVAPGS
(k)	PI3K p85	PAPALPPKPPK
(l)	AFAP-110	PPDNGPPPLPTSS
(m)	AFAP-110	PPQMPLPEIQQW
(n)	3BP-1	APTMPPPLPPVPP
(o)	3BP-2	FPAYPPPPVPVP

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FIG. 18.

```

V      1      11      21      31      41      51
      MTTSNVELIP IYTDWANRHL SKGSLSKSIR DISNDFRDYR LVSQLINVIV PINEFSPAFT
-----
H      1      11      21      31      41      51
V      61      71      81      91      101      111
      KRLAKITSNL DGLETCLDYL KNGLDCSKL TKTDIDSGNL GAVLQLLFLF STYKQKLRQL
-----
H      61      71      81      91      101      111
V      121     131     141     151     161     171
      KKDQKKLEQL PTSIMPPAVS KLPSPRVATS ATASATNPNS NFPQMSTSR L QTPQSRISKI
-----
H      121     131     141     151     161     171
V      181     191     201     211     221     231
      DSSKIGIKPK TSGLKPPSSS TTSSNNTNSF RPSSRSSGNN NVGSTISTSA KSLESSSTYS
-----
H      181     191     201     211     221     231
V      241     251     261     271     281     291
      SISNLNRPTS QLQKPSRPQT QLV RVATTTK IGSSKLAAPK AVSTPKLASV KTIGAKQEPD
-----
H      241     251     261     271     281     291
V      301     311     321     331     341     351
      NSGGGGGGML KLKLFSSKNP SSSSNSPQPT RKA AAVPQQQ TSKIAAPVK SGLKPPTS KL
      ..
      -----ML KLKLFSSKNP SSSSNSPQPT RKA AAVPQQQ TSKIAAPVK SGLKPPTS KL
H      301     311     321     331     341     351
V      361     371     381     391     401     411
      GSATSMKLC TPKVSYRKTD APIISQQDSK RCSKSSEES GYAGFNSTSP TSSSTEGSLS
      ..
      GSATSMKLC TPKVSYRKTD APIISQQDSK RCSKSSEES GYAGFNSTSP TSSSTEGSLS
H      361     371     381     391     401     411
V      421     431     441     451     461     471
      MHSTSSKSST SDEKSPSSDD LTLNASIVTA IRQPIAATPV SPNIINKPVE EKPTLAVKGV
      ..
      MHSTSSKSST SDEKSPSSDD LTLNASIVTA IRQPIAATPV SPNIINKPVE EKPTLAVKGV
H      421     431     441     451     461     471
V      481     491     501     511     521     531
      KSTAKDPPP AVPPRDTQPT IGVVSPIMAH KKLTNDPVIS EKPEPEKLQS MSIDTTDVPP
      ..
      KSTAKDPPP AVPPRDTQPT IGVVSPIMAH KKLTNDPVIS EKPEPEKLQS MSIDTTDVPP
H      481     491     501     511     521     531
V      541     551     561     571     581     591
      LPPLKSVVPL KMTSIRQPPT YDVLLKQGKI TSPVKSFGYE QSSASEDSIV AHASAQVTPP
      ..
      LPPLKSVVPL KMTSIRQPPT YDVLLKQGKI TSPVKSFGYE QSSASEDSIV AHASAQVTPP
H      541     551     561     571     581     591
V      601     611     621     631     641     651
      TKTSGNHSLE RRMGKNKTSE SSGYTS DAGV AMCAKMKREKL KEYDDMTRRA QNGYPDNFED
      ..
      TKTSGNHSLE RRMGKNKTSE SSGYTS DAGV AMCAKMKREKL KEYDDMTRRA QNGYPDNFED
H      601     611     621     631     641     651
V      661     671     681     691     701     711
      SSSLSSGISD NNELDDISTD DLSGVDMATV ASKHS DYSHF VRHPTSSSK PRVPSRSSTS
      ..
      SSSLSSGISD NNELDDISTD DLSGVDMATV ASKHS DYSHF VRHPTSSSK PRVPSRSSTS
H      661     671     681     691     701     711
V      721     731     741     751     761     771
      VDSRSRAEQE NVYKLLSQCR TSQRGAAATS TFGQHS LRSP GYSSYPHLS VSADKDTMSM
      ..

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FIG. 18 CONTINUED.

	VDSRSRAEQE	NVYKLLSQCR	TSQRGAAATS	TFGQHSRLSP	GYSSYSPLHS	VSADKDTMSM
H	721	731	741	751	761	771
V	781	791	801	811	821	831
	HSQTSRRPSS	QKPSYSGQFH	SLDRKCHLQE	FTSTEHRMAA	LLSPRRVPNS	MSKYDSSGSY
	.....	.....	.....	.....	.....	.....
	HSQTSRRPSS	QKPSYSGQFH	SLDRKCHLQE	FTSTEHRMAA	LLSPRRVPNS	MSKYDSSGSY
H	781	791	801	811	821	831
V	841	851	861	871	881	891
	SARSRGGSST	GIYGETFQLH	RLSDEKSPAH	SAKSEMGSOL	SLASTTAYGS	LNEKYEHAIR
	.....	.....	.....	.....	.....	.....
	SARSRGGSST	GIYGETFQLH	RLSDEKSPAH	SAKSEMGSOL	SLASTTAYGS	LNEKYEHAIR
H	841	851	861	871	881	891
V	901	911	921	931	941	951
	DMARDLECYK	NTVDSLTKKQ	ENYGALFDLF	EQKLRKLTQH	IDRSNLKPEE	AIRFRQDIAH
	.....	.....	.....	.....	.....	.....
	DMARDLECYK	NTVDSLTKKQ	ENYGALFDLF	EQKLRKLTQH	IDRSNLKPEE	AIRFRQDIAH
H	901	911	921	931	941	951
V	961	971	981	991	1001	1011
	LRDISNHLAS	NSAHANEGAG	ELLRQPSLES	VASHRSSMSS	SSKSSKQEKI	SLSSFGKNKK
	.....	.....	.....	.....	.....	.....
	LRDISNHLAS	NSAHANEGAG	ELLRQPSLES	VASHRSSMSS	SSKSSKQEKI	SLSSFGKNKK
H	961	971	981	991	1001	1011
V	1021	1031	1041	1051	1061	1071
	SWIRSSLSKF	TKKKKNKYDE	AHMPSISGSQ	GTLDNIDVIE	LKQELKERDS	ALYEVRLDNL
	.....	.....	.....	.....	.....	.....
	SWIRSSLSKF	TKKKKNKYDE	AHMPSISGSQ	GTLDNIDVIE	LKQELKERDS	ALYEVRLDNL
H	1021	1031	1041	1051	1061	1071
V	1081	1091	1101	1111	1121	1131
	DRAREVDVLR	ETVNKLKTEN	KQLKKEVDKL	TNGPATRASS	RASIPVIYDD	EHVYDAACSS
	.....	.....	.....	.....	.....	.....
	DRAREVDVLR	ETVNKLKTEN	KQLKKEVDKL	TNGPATRASS	RASIPVIYDD	EHVYDAACSS
H	1081	1091	1101	1111	1121	1131
V	1141	1151	1161	1171	1181	1191
	TSASQSSKRS	SGCNSIKVTV	NVDIAGEISS	IVNPDKEIIV	GYLAMSTSQS	CWKDIDVSIL
	.....	.....	.....	.....	.....	.....
	TSASQSSKRS	SGCNSIKVTV	NVDIAGEISS	IVNPDKEIIV	GYLAMPTSQS	CWKDIDVSIL
H	1141	1151	1161	1171	1181	1191
V	1201	1211	1221	1231	1241	1251
	GLFEVYLSRI	DVEHQLGIDA	RDSILGYQIG	ELRRVIGDST	TMITSHPTDI	LTSSTTIRMF
	.....	.....	.....	.....	.....	.....
	GLFEVYLSRI	DVEHQLGIDA	RDSILGYQIG	ELRRVIGDST	TMITSHPTDI	LTSSTTIRMF
H	1201	1211	1221	1231	1241	1251
V	1261	1271	1281	1291	1301	1311
	MHGAAQSRVD	SLVLDMLLPK	QMILQLVKSI	LTERRLVLAG	ATGIGKSKLA	KTAAAYVSIR
	.....	.....	.....	.....	.....	.....
	MHGAAQSRVD	SLVLDMLLPK	QMILQLVKSI	LTERRLVLAG	ATGIGKSKLA	KTAAAYVSIR
H	1261	1271	1281	1291	1301	1311
V	1321	1331	1341	1351	1361	1371
	TNQSEDSIVN	ISIPENNKEE	LLQVERRLEK	ILRSKESCIV	ILDNIPKNRI	AFVVSVFANV
	.....	.....	.....	.....	.....	.....
	TNQSEDSIVN	ISIPENNKEE	LLQVERRLEK	ILRSKESCIV	ILDNIPKNRI	AFVVSVFANV
H	1321	1331	1341	1351	1361	1371
V	1381	1391	1401	1411	1421	1431
	PLQNEGPFV	VCTVNRYQIP	ELQIHNNFKM	SVMSNRLEGF	ILRYLRRRAV	EDEYRLTVQM
	.....	.....	.....	.....	.....	.....
	PLQNEGPFV	VCTVNRYQIP	ELQIHNNFKM	SVMSNRLEGF	ILRYLRRRAV	EDEYRLTVQM
H	1381	1391	1401	1411	1421	1431
V	1441	1451	1461	1471	1481	1491
	PSELFKIIDF	FPIALQAVNN	FIEKTNVSDV	TVGPRACLNC	PLTVDGSREW	FIRLWNNENFI
	.....	.....	.....	.....	.....	.....

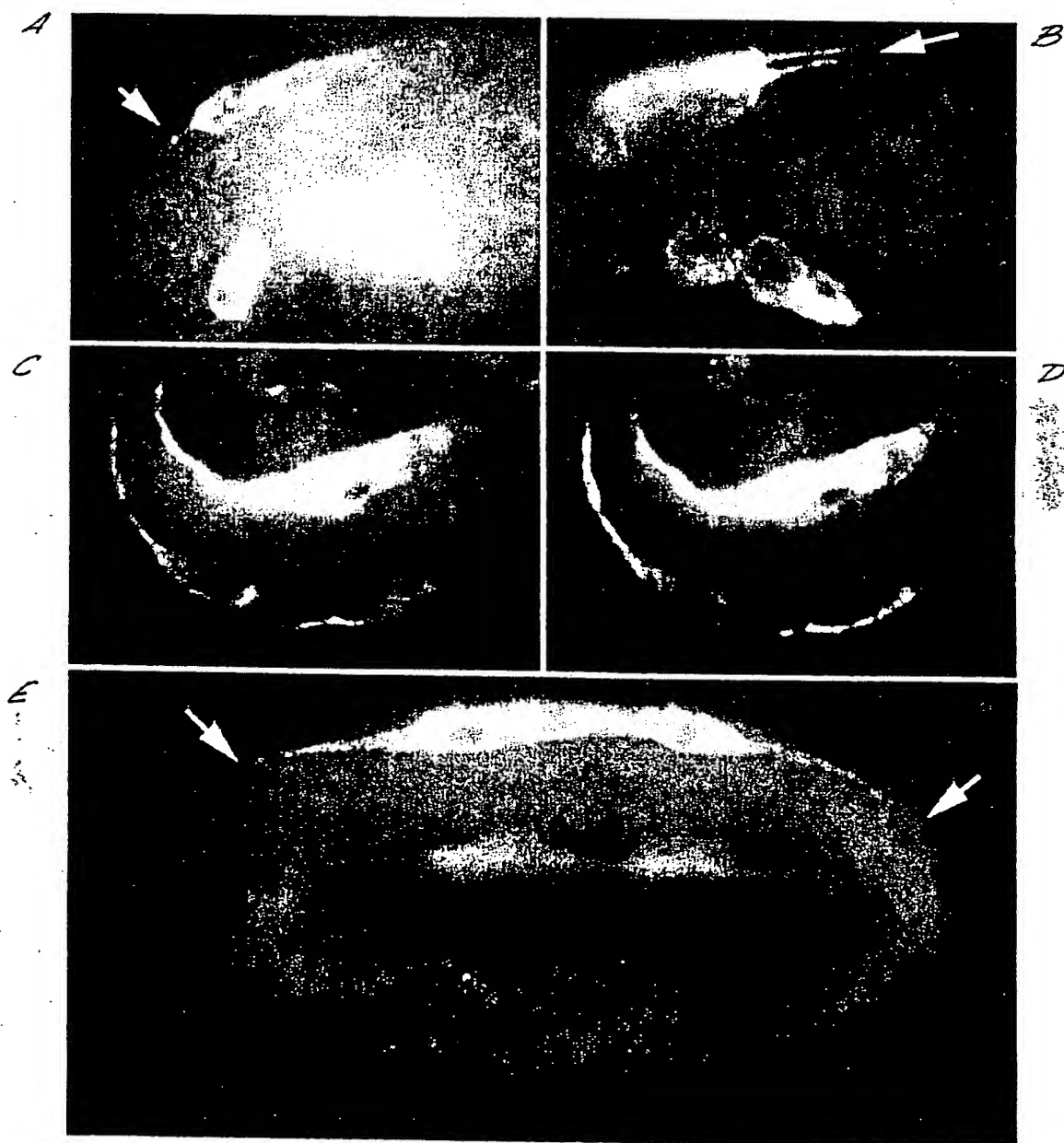
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## FIG. 18 CONTINUED

		PSELFKIIDF	FPIALQAVNN	FIEKTNVSDV	TVGPRACLNC	PLTVDGSREW	FIRLWNENFI
H	1441	1451	1461	1471	1481	1491	
V	1501	1511	1521	1531	1541	1551	
		PYLERVARDG KKNLRSLHFL RGSRRHRL-- -----					
		.....					
		PYLERVARDG	KKTFGRCTSF	EDPTDIVSEK	WPWFDGENPE	NVLKRLQLQD	LVPSPANSSR
H	1501	1511	1521	1531	1541	1551	
V		-----					
		QHFNPLESLI QLHATKHQTI DNI					
H	1561	1571	1581				

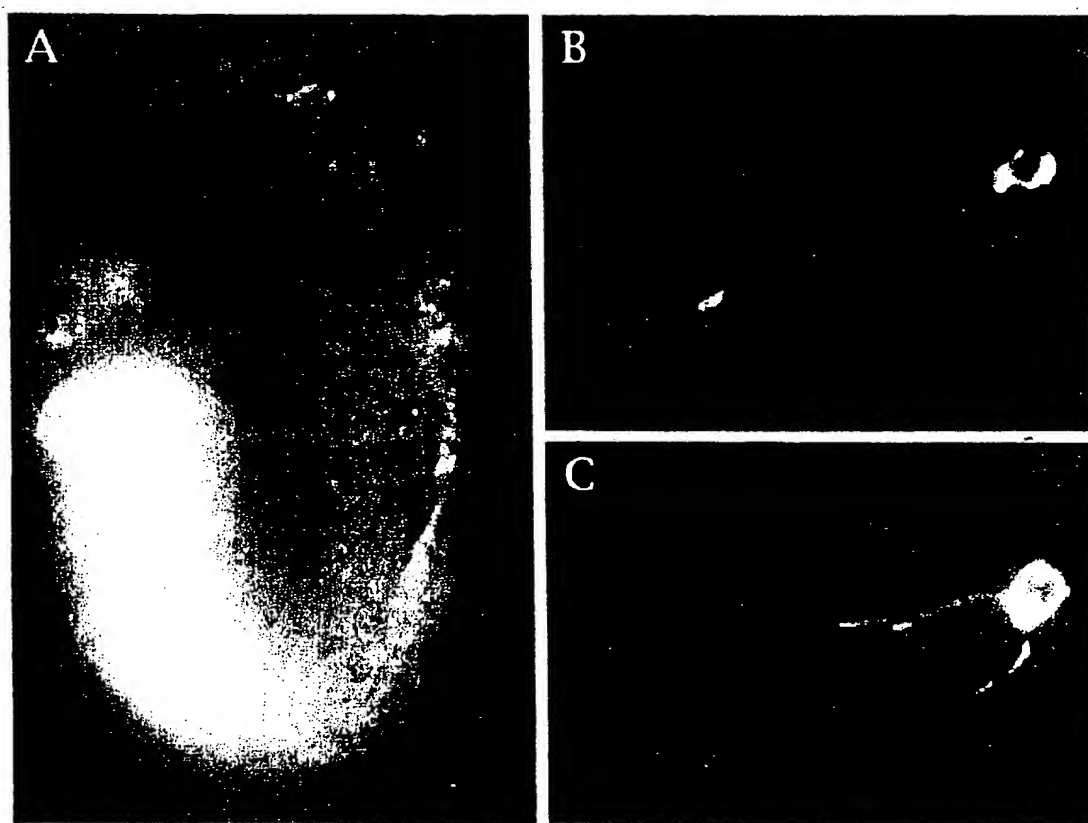
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FIG. 19.



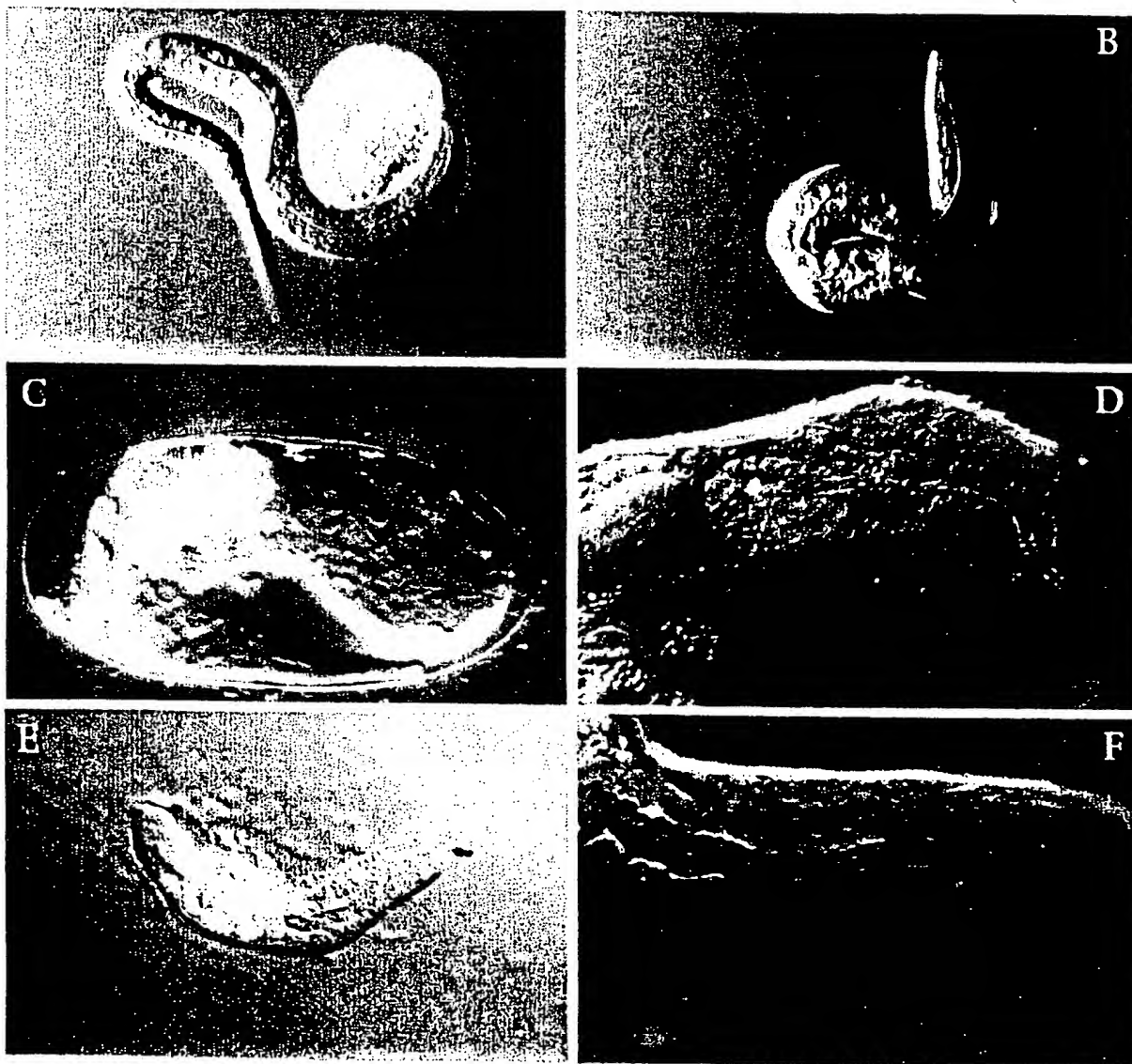
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*FIG. 20.*



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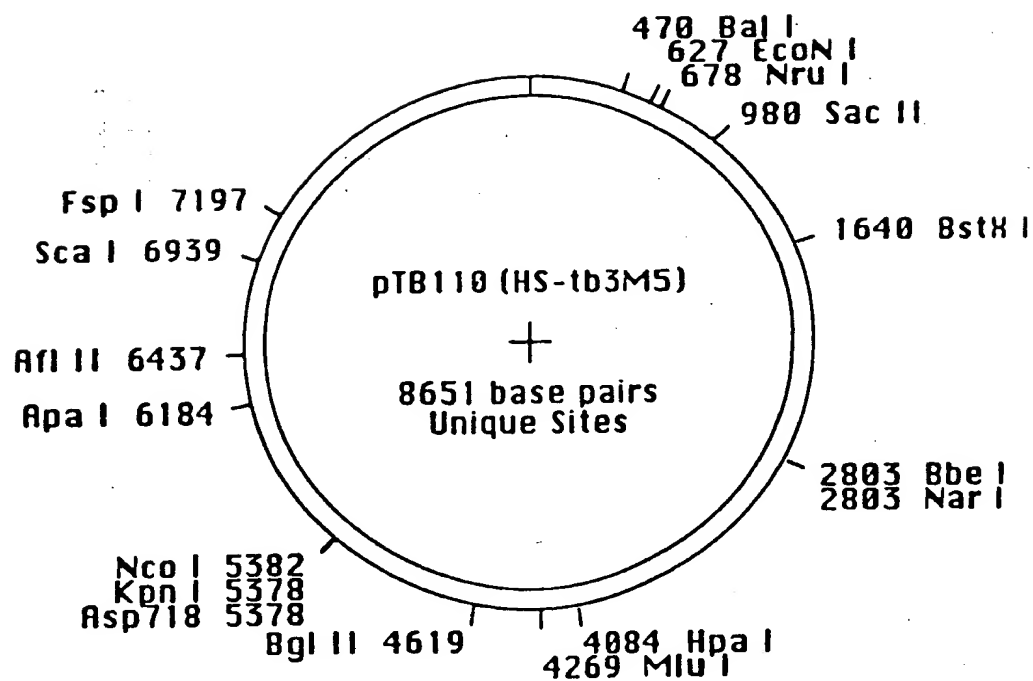
*FIG. 21.*





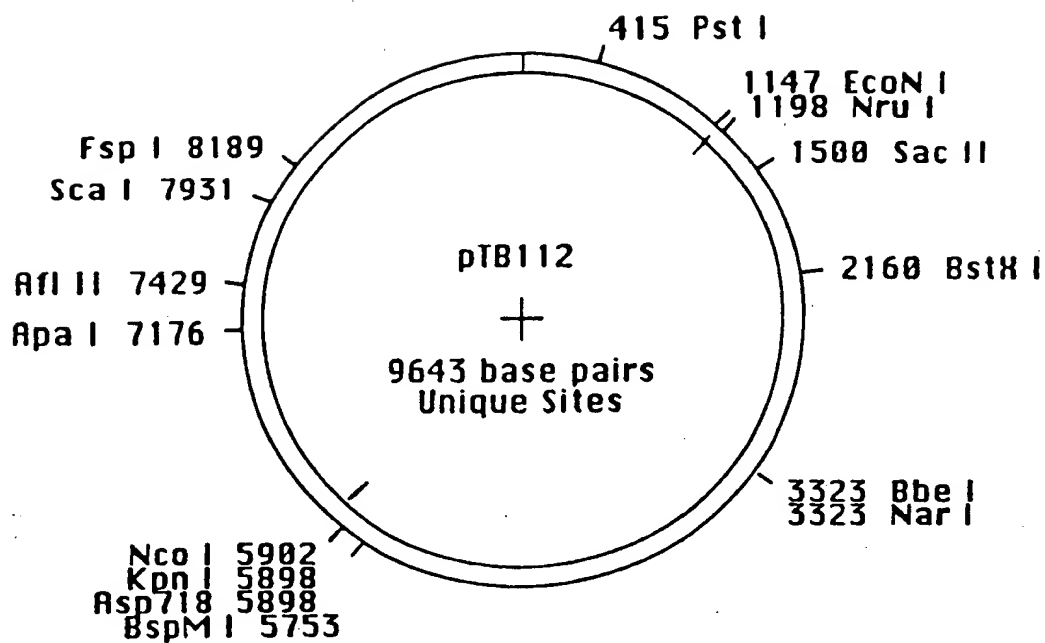
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FIG. 22.



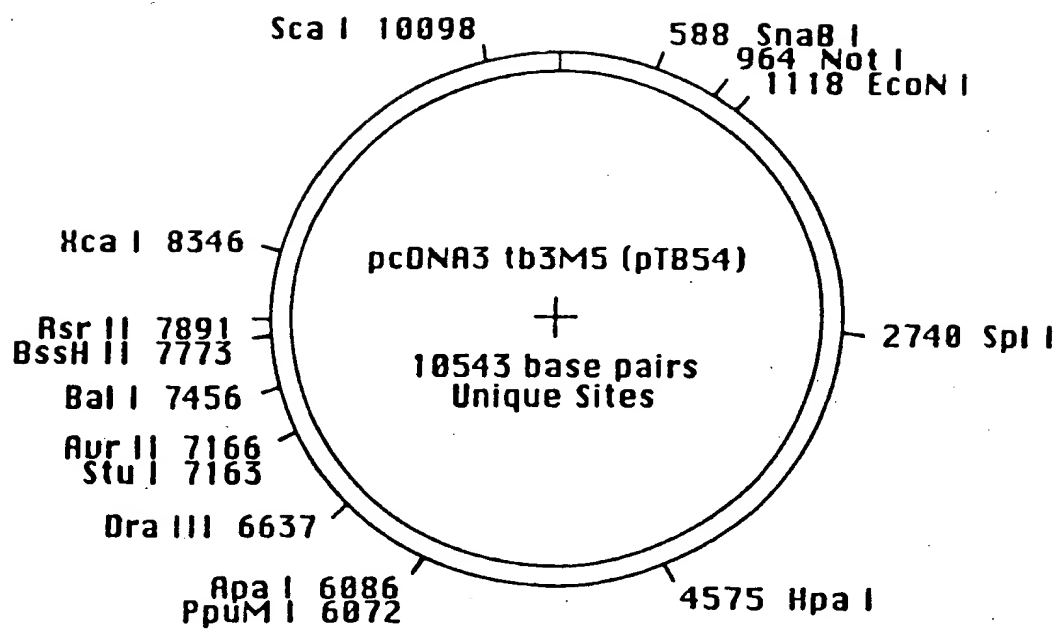
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FIG. 23.



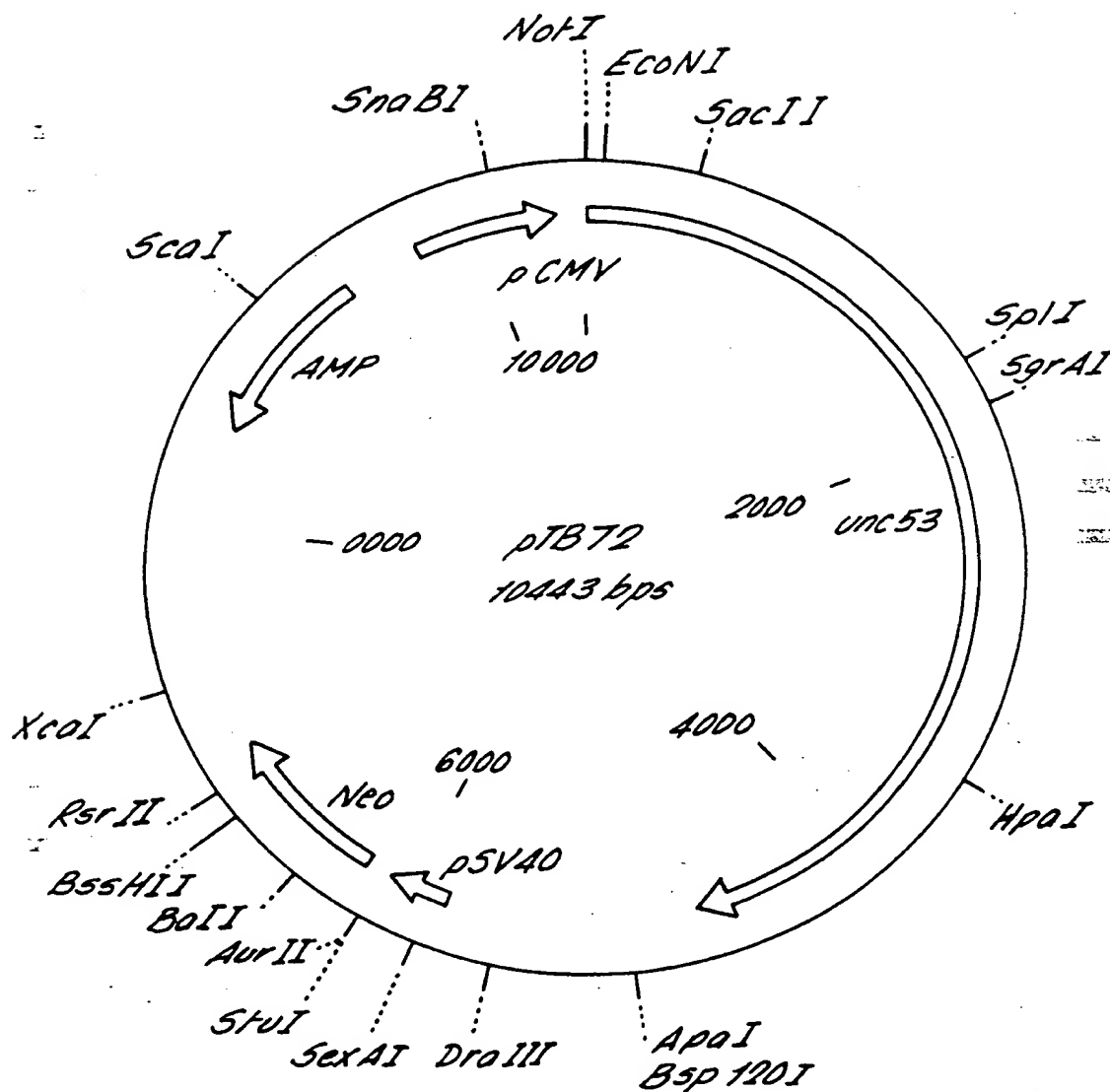
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FIG. 24.



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FIG. 25.



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FIG. 26.

GGCCGCCGCC	ATGACGACGT	CAAATGTAGA	ATTGATACCA	ATCTACACGG	ATTGGGCCAA	60
TCGGCACCTT	TCGAAGGGCA	GCTTATCAAA	GTCGATTAGG	GATATTTCCA	ATGATTTTCG	120
CGACTATCGA	CTGGTTTCTC	AGCTTATTAA	TGTGATCGTT	CCGATCAACG	AATTCTCGCC	180
TGCATTACAG	AAACGTTTGG	CAAAAATCAC	ATCGAACCTG	GATGGCCTCG	AAACGTGTCT	240
CGACTACCTG	AAAAATCTGG	GTCTCGACTG	CTCGAACTC	ACCAAAACCG	ATATCGACAG	300
CGGAAACTTG	GGTGCA GTTC	TCCAGCTGCT	CTTCCTGCTC	TCCACCTACA	AGCAGAAGCT	360
TCGGCAACTG	AAAAAAGATC	AGAAGAAATT	GGAGCAACTA	CCCACATCCA	TTATGCCACC	420
CGCGGTTTCT	AAATTACCCT	CGCCACGTGT	CGCCACGTCA	GCAACCGCTT	CAGCAACTAA	480
CCCAAATTCC	AACTTTCCAC	AAATGTCAAC	ATCCAGGCTT	CAGACTCCAC	AGTCAAGAAT	540
ATCGAAAATT	GATTCATCAA	AGATTGGTAT	CAAGCCAAAG	ACGTCTGGAC	TTAAACCACC	600
CTCATCATCA	ACCACTTCAT	CAAATAATAC	AAATTCAATC	CGTCCGTCGA	GCCGTTCGAG	660
TGGCAATAAT	AATGTTGGCT	CGACGATATC	CACATCTGCG	AAGAGCTTAG	AATCATCATC	720
AACGTACAGC	TCTATTTCTG	ATCTAAACCG	ACCTACCTCC	CAACTCCAAA	AACCTTCTAG	780
ACCACAAACC	CAGCTAGTTC	GTGTTGCTAC	AACTACAAA	ATCGGAAGCT	CAAAGCTAGC	840
CGCTCCGAAA	GCCGTGAGCA	CCCCAAAAC	TGCTTCTGTG	AAGACTATTG	GAGCAAAACA	900
AGAGCCCGAT	AACAGCGGTG	GTGGTGGTGG	TGGAATGCTG	AAATTAAAGT	TATTCAGTAG	960
CAAAAACCCA	TCTTCCTCAT	CGAATAGCCC	ACAACCTACG	AGAAAGGCGG	CGGCGGTGCC	1020
TCAACAACAA	ACTTTGTCTG	AAATCGCTGC	CCCAGTGAAA	AGTGGCCTGA	AGCCGCCGAC	1080
CAGTAAGCTG	GGAAGTGCCA	CGTCTATGTC	GAAGCTTTGT	ACGCCAAAAG	TTTCCTACCG	1140
TAAACGGAC	GCCCCAATCA	TATCTCAACA	AGACTCGAAA	CGATGCTCAA	AGAGCAGTGA	1200
AGAAGAGTCC	GGATACGCTG	GATTCAACAG	CACGTCGCCA	ACGTCATCAT	CGACGGAAGG	1260
TTCCCTAAGC	ATGCATTCCA	CATCTTCCAA	GAGTTCAACG	TCAGACGAAA	AGTCTCCGTC	1320
ATCAGACGAT	CTTACTCTTA	ACGCCTCCAT	CGTGACAGCT	ATCAGACAGC	CGATAGCCGC	1380
AACACCGGTT	TCTCCAAATA	TTATCAACAA	GCCTGTTGAG	GAAAAACCAA	CACTGGCAGT	1440

*FIG. 26 CONTINUED.**54/99*

GAAAGGAGTG AAAAGCACAG CGAAAAAAGA TCCACCTCCA GCTGTTCCGC CACGTGACAC	1500
CCAGCCAACA ATCGGAGTTG TTAGTCCAAT TATGGCACAT AAGAAGTTGA CAAATGACCC	1560
CGTGATATCT GAAAAACCAG AACCTGAAAA GCTCCAATCA ATGAGCATCG ACACGACGGA	1620
CGTTCCACCG CTTCACCTC TAAAATCAGT TGTTCCACTT AAAATGACTT CAATCCGACA	1680
ACCACCAACG TACGATGTTT TTCTAAAACA AGGAAAAATC ACATCGCCTG TCAAGTCGTT	1740
TGGATATGAG CAGTCGTCCG CGTCTGAAGA CTCCATTGTG GCTCATGCGT CGGCTCAGGT	1800
GACTCCGCCG ACAAAAACTT CTGGTAATCA TTCGCTGGAG AGAAGGATGG GAAAGAATAA	1860
GACATCAGAA TCCAGCGGCT ACACCTCTGA CGCCGGTGTT GCGATGTGCG CCAAAATGAG	1920
GGAGAAGCTG AAAGAATACG ATGACATGAC TCGTCGAGCA CAGAACGGCT ATCCTGACAA	1980
CTTCGAAGAC AGTTCCTCCT TGTCGTCTGG AATATCCGAT AACAACGAGC TCGACGACAT	2040
ATCCACGGAC GATTTGTCCG GAGTAGACAT GGCAACAGTC GCCTCCAAAC ATAGCGACTA	2100
TTCCCACTTT GTTCGCCATC CCACGTCTTC TTCCTCAAAG CCCCAGAGTCC CCAGTCGGTC	2160
CTCCACATCA GTCGATTCTC GATCTCGAGC AGAACAGGAG AATGTGTACA AACTTCTGTC	2220
CCAGTGCCGA ACGAGCCAAC GTGGCGCCGC TGCCACCTCA ACCTTCGGAC AACATTGCGT	2280
AAGATCCCCG GGATACTCAT CCTATTCTCC ACACTTATCA GTGTCAGCTG ATAAGGACAC	2340
AATGTCTATG CACTCACAGA CTAGTCGACG ACCTTCTTCA CAAAAACCAA GCTATTCAGG	2400
CCAATTTTAT TCACTTGATC GTAAATGCCA CCTTCAAGAG TTCACATCCA CCGAGCACAG	2460
AATGGCGGCT CTCTTGAGCC CGAGACGGGT GCCGAAGTCG ATGTCGAAAT ATGATTCTTC	2520
AGGATCCTAC TCGGCGCGTT CCCGAGGTGG AAGCTCTACT GGTATCTATG GAGAGACGTT	2580
CCAAGTGCAC AGACTATCCG ATGAAAAATC CCCCACACAT TCTGCCAAAA GTGAGATGGG	2640
ATCCCAACTA TCACTGGCTA GCACGACAGC ATATGGATCT CTCAATGAGA AGTACGAACA	2700
TGCTATTCTG GACATGGCAC GTGACTTGGA GTGTTACAAG AACACTGTGCT ACTCACTAAC	2760
CAAGAAACAG GAGAACTATG GAGCATTGTT TGATCTTTTT GAGCAAAAGC TTAGAAAACT	2820
CACTCAACAC ATTGATCGAT CCAACTTGAA GCCTGAAGAG GCAATACGAT TCAGGCAGGA	2880
CATTGCTCAT TTGAGGGATA TTAGCAATCA TCTTGCATCC AACTCAGCTC ATGCTAACGA	2940
AGGCGCTGGT GAGCTTCTTC GTCAACCATC TCTGGAATCA GTTGCATCCC ATCGATCATC	3000
GATGTCATCG TCGTCGAAAA GCAGCAAGCA GGAGAAGATC AGCTTGAGCT CGTTTGCCAA	3060
GAACAAGAAG AGCTGGATCC GCTCCTCACT CTCCAAGTTC ACCAAGAAGA AGAACAAGAA	3120
CTACGACGAA GCACATATGC CATCAATTTT CGGATCTCAA GGAAGTCTTG ACAACATTGA	3180
TGTGATTGAG TTGAAGCAAG AGCTCAAAGA ACGCGATAGT GCACTTTACG AAGTCCGCCT	3240
TGACAATCTG GATCGTGCCC GCGAAGTTGA TGTTCTGAGG GAGACAGTGA ACAAGTTGAA	3300
AACCGAGAAC AAGCAATTAA AGAAAGAAGT GGACAAACTC ACCAACGGTC CAGCCACTCG	3360

*FIG. 26 CONTINUED.**55/99*

TGCTTCTTCC CGCGCCTCAA TTCCAGTTAT CTACGACGAT GAGCATGTCT ATGATGCAGC	3420
GTGTAGCAGT ACATCAGCTA GTCAATCTTC GAAACGATCC TCTGGCTGCA ACTCAATCAA	3480
GGTTACTGTA AACGTGGACA TCGCTGGAGA AATCAGTTCG ATCGTTAACC CGGACAAAAGA	3540
GATAATCGTA GGATATCTTG CCATGTCAAC CAGTCAGTCA TGCTGGAAAAG ACATTGATGT	3600
TTCTATTCTA GGACTATTTG AAGTCTACCT ATCCAGAATT GATGTGGAGC ATCAACTTGG	3660
AATCGATGCT CGTGATTCTA TCCTTGGCTA TCAAATTGGT GAACTTCGAC GCGTCATTGG	3720
AGACTCCACA ACCATGATAA CCAGCCATCC AACTGACATT CTTACTTCCT CAACTACAAT	3780
CCGAATGTTT ATGCACGGTG CCGCACAGAG TCGCGTAGAC AGTCTGGTCC TTGATATGCT	3840
TCTTCCAAAG CAAATGATTC TCCAACCTCGT CAAGTCAATT TTGACAGAGA GACGTCTGGT	3900
GTTAGCTGGA GCAACTGGAA TTGGAAAGAG CAACTGGCG AAGACCCTGG CTGCTTATGT	3960
ATCTATTCGA ACAAATCAAT CCGAAGATAG TATTGTAAAT ATCAGCATT C TGAAAACAA	4020
TAAAGAAGAA TTGCTTCAAG TGGAACGACG CCTGGAAAAG ATCTTGAGAA GCAAAGAATC	4080
ATGCATCGTA ATTCTAGATA ATATCCCAA GAATCGAATT GCATTGTGTG TATCCGTTTT	4140
TGCAAATGTC CCACTTCAA ACAACGAAGG TCCATTTGTA GTATGCACAG TCAACCGATA	4200
TCAAATCCCT GAGCTTCAA TTCACCACAA TTTCAAATG TCAGTAATGT CGAATCGTCT	4260
CGAAGGATTC ATCCTACGTT ACCTCCGACG ACGGGCGGTA GAGGATGAGT ATCGTCTAAC	4320
TGTACAGATG CCATCAGAGC TCTTCAAAT CATTGACTTC TTCCAATAG CTCTTCAGGC	4380
CGTCAATAAT TTTATTGAGA AAACGAATTC TGTTGATGTG ACAGTTGGTC CAAGAGCATG	4440
CTTGAAGTGT CCTCTAACTG TCGATGGATC CCGTGAATGG TTCATTCGAT TGTGGAATGA	4500
GAACTTCATT CCATATTTGG AACGTGTTGC TAGAGATGGC AAAAAACCT TCGGTCGCTG	4560
CACTTCCTTC GAGGATCCCA CCGACATCGT CTCTAAAAA TGGCCGTGGT TCGATGGTGA	4620
AAACCCGGAG AATGTGCTCA AACGTCTTCA ACTCCAAGAC CTCGTCCCGT CACCTGCCAA	4680
CTCATCCCGA CAACACTTCA ATCCCTCGA GTCGTTGATC CAATTGCATG CTACCAAGCA	4740
TCAGACCATC GACAACATTT GAACAGAAGA CTCTAATCTT CTCTCGCCTC TCCCCGCTT	4800
TCCTTATCTT CGTACCGGTA CCTGATGATT CCCCATTTTC CCCCTTTTCC CCCCAATTC	4860
CCAGAACCTC CTGTTCCCTT TGTTCCTAGT CCTCCCGGGT GCCGACGCCG AAGCGATTGA	4920
AAAACCTTTT TCTTTCCGAA ACATTTCCTA TTGCTCATT ATAGTCAAAT TGAATAAACA	4980
GTGTATGTAC TTAACAAAAA AAAAAAATA ACTCGAGGGG GGGCCCTATT CTATAGTGTC	5040
ACCTAAATGC TAGAGCTCGC TGATCAGCCT CGACTGTGCC TTCTAGTTGC CAGCCATCTG	5100
TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCCTTT	5160
CCTAATAAAA TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCATTCT ATTCTGGGGG	5220
GTGGGGTGGG GCAGGACAGC AAGGGGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG	5280

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## FIG. 26 CONTINUED.

ATGCGGTGGG CTCTATGGCT TCTGAGGCGG AAAGAACCAG CTGGGGCTCT AGGGGGTATC	5340
CCCACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT GGTGGTTACG CGCAGCGTGA	5400
CCGCTACACT TGCCAGCGCC CTAGCGCCCG CTCCTTTCGC TTTCTTCCCT TCCTTCTCG	5460
CCACGTTTCG CGGCTTTCCC CGTCAAGCTC TAAATCGGGG CATCCCTTTA GGGTTCCGAT	5520
TTAGTGCTTT ACGGCACCTC GACCCCAAAA AACTTGATTA GGGTGATGGT TCACGTAGTG	5580
GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT GGAGTCCACG TTCTTTAATA	5640
GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACCCTAT CTCGGTCTAT TCTTTTGATT	5700
TATAAGGGAT TTTGGGGATT TCGGCCTATT GGTAAAAAA TGAGCTGATT TAACAAAAAT	5760
TTAACGCGAA TTAATTCTGT GGAATGTGTG TCAGTTAGGG TGTGGAAAGT CCCCAGGCTC	5820
CCCAGGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC AGGTGTGGAA	5880
AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA	5940
CCATAGTCCC GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT	6000
CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC GCCTCTGCCT	6060
CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTGGAGG CCTAGGCTTT TGCAAAAAGC	6120
TCCCGGGAGC TTGTATATCC ATTTTCGGAT CTGATCAAGA GACAGGATGA GGATCGTTTC	6180
GCATGATTGA ACAAGATGGA TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT	6240
TCGGCTATGA CTGGGCACAA CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT	6300
CAGCGCAGGG GCGCCCGGT CTTTTTGTC AAGACCGACCT GTCCGGTGCC CTGAATGAAC	6360
TGCAGGACGA GGCAGCGCG CTATCGTGGC TGGCCACGAC GGGCGTTCCT TGCGCAGCTG	6420
TGCTCGACGT TGTCACTGAA GCGGGAAGGG ACTGGCTGCT ATTGGGCGAA GTGCCGGGGC	6480
AGGATCTCCT GTCATCTCAC CTTGCTCCTG CCGAGAAAGT ATCCATCATG GCTGATGCAA	6540
TGCGGCGGCT GCATACGCTT GATCCGGCTA CCTGCCCATT CGACCACCAA GCGAAACATC	6600
GCATCGAGCG AGCACGTACT CGGATGGAAG CCGGTCTTGT CGATCAGGAT GATCTGGACG	6660
AAGAGCATCA GGGGCTCGCG CCAGCCGAAC TGTTCCGCAG GCTCAAGGCG CGCATGCCCG	6720
ACGGCGAGGA TCTCGTCGTG ACCCATGGCG ATGCCTGCTT GCCGAATATC ATGGTGGAAG	6780
ATGGCCGCTT TTCTGGATT C ATCGACTGTG GCCGGCTGGG TGTGGCGGAC CGCTATCAGG	6840
ACATAGCGTT GGCTACCCGT GATATTGCTG AAGAGCTTGG CGGCGAATGG GCTGACCGCT	6900
TCCTCGTGCT TTACGGTATC GCCGCTCCCG ATTCGCAGCG CATCGCCTTC TATCGCCTTC	6960
TTGACGAGTT CTTCTGAGCG GGACTCTGGG GTTCGAAATG ACCGACCAAG CGACGCCCAA	7020
CCTGCCATCA CGAGATTTCG ATTCCACCGC CGCCTTCTAT GAAAGGTTGG GCTTCGGAAT	7080
CGTTTCCGG GACGCCGGCT GGATGATCCT CCAGCGCGGG GATCTCATGC TGGAGTTCTT	7140
CGCCCACCCC AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC	7200



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FIG. 26 CONTINUED.

AAATTTTACA	AATAAAGCAT	TTTTTTCAC	TGATTCTAGT	TGTGGTTTGT	CCAAACTCAT	7260
CAATGTATCT	TATCATGTCT	GTATACCGTC	GACCTCTAGC	TAGAGCTTGG	CGTAATCATG	7320
GTCATAGCTG	TTTCCTGTGT	GAAATTGTTA	TCCGCTCACA	ATTCCACACA	ACATACGAGC	7380
CGGAAGCATA	AAGTGTAAG	CCTGGGGTGC	CTAATGAGTG	AGCTAACTCA	CATTAATTGC	7440
GTTGCGCTCA	CTGCCCCTT	TCCAGTCGGG	AAACCTGTCG	TGCCAGCTGC	ATTAATGAAT	7500
CGGCCAACGC	GCGGGGAGAG	GCGGTTTGCG	TATTGGGCGC	TCTTCCGCTT	CCTCGCTCAC	7560
TGACTCGCTG	CGCTCGGTG	TTCGGCTGCG	GCGAGCGGTA	TCAGCTCACT	CAAAGGCGGT	7620
AATACGGTTA	TCCACAGAAT	CAGGGGATAA	CGCAGGAAAG	AACATGTGAG	CAAAAGGCCA	7680
GCAAAAGGCC	AGGAACCGTA	AAAAGGCCGC	GTTGCTGGCG	TTTTTCCATA	GGCTCCGCCC	7740
CCCTGACGAG	CATCACAAAA	ATCGACGCTC	AAGTCAGAGG	TGGCGAAACC	CGACAGGACT	7800
ATAAAGATAC	CAGGCGTTTC	CCCCTGGAAG	CTCCCTCGTG	CGCTCTCCTG	TTCCGACCCCT	7860
GCCGCTTACC	GGATACCTGT	CCGCTTTTCT	CCCTTCGGGA	AGCGTGGCGC	TTTCTCAATG	7920
CTCACGCTGT	AGGTATCTCA	GTTGCGGTGA	GGTCGTTTCG	TCCAAGCTGG	GCTGTGTGCA	7980
CGAACCCCCC	GTTGAGCCCG	ACCGCTGCGC	CTTATCCGGT	AACTATCGTC	TTGAGTCCAA	8040
CCCGGTAAGA	CACGACTTAT	CGCCACTGGC	AGCAGCCACT	GGTAACAGGA	TTAGCAGAGC	8100
GAGGTATGTA	GGCGGTGCTA	CAGAGTTCTT	GAAGTGGTGG	CCTAACTACG	GCTACACTAG	8160
AAGGACAGTA	TTTGGTATCT	GCGCTCTGCT	GAAGCCAGTT	ACCTTCGGAA	AAAGAGTTGG	8220
TAGCTCTTGA	TCCGGCAAAC	AAACCACCGC	TGGTAGCGGT	GGTTTTTTTG	TTTGCAAGCA	8280
GCAGATTACG	CGCAGAAAAA	AAGGATCTCA	AGAAGATCCT	TTGATCTTTT	CTACGGGGTC	8340
TGACGCTCAG	TGGAACGAAA	ACTCACGTGA	AGGGATTTTG	GTCATGAGAT	TATCAAAAAG	8400
GATCTTCACC	TAGATCCTTT	TAAATTAAAA	ATGAAGTTTT	AAATCAATCT	AAAGTATATA	8460
TGAGTAAACT	TGGTCTGACA	GTTACCAATG	CTTAATCAGT	GAGGCACCTA	TCTCAGCGAT	8520
CTGTCTATTT	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC	GTGTAGATAA	CTACGATACG	8580
GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC	AATGATACCG	CGAGACCCAC	GCTCACCGGC	8640
TCCAGATTTA	TCAGCAATAA	ACCAGCCAGC	CGGAAGGGCC	GAGCGCAGAA	GTGGTCTTGC	8700
AACTTTATCC	GCCTCCATCC	AGTCTATTAA	TTGTTGCCGG	GAAGCTAGAG	TAAGTAGTTC	8760
GCCAGTTAAT	AGTTTGCGCA	ACGTTGTTGC	CATTGCTACA	GGCATCGTGG	TGTCACGCTC	8820
GTCGTTTGGT	ATGGCTTCAT	TCAGCTCCGG	TTCCCAACGA	TCAAGGCGAG	TTACATGATC	8880
CCCCATGTTG	TGCAAAAAAG	CGGTTAGCTC	CTTCGGTCCT	CCGATCGTTG	TCAGAAGTAA	8940
GTTGGCCGCA	GTGTTATCAC	TCATGGTTAT	GGCAGCACTG	CATAATTCTC	TTACTGTCAT	9000
GCCATCCGTA	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA	ACCAAGTCAT	TCTGAGAATA	9060
GTGTATGCGG	CGACCGAGTT	GCTCTTGCCC	GGCGTCAATA	CGGGATAATA	CCGCGCCACA	9120

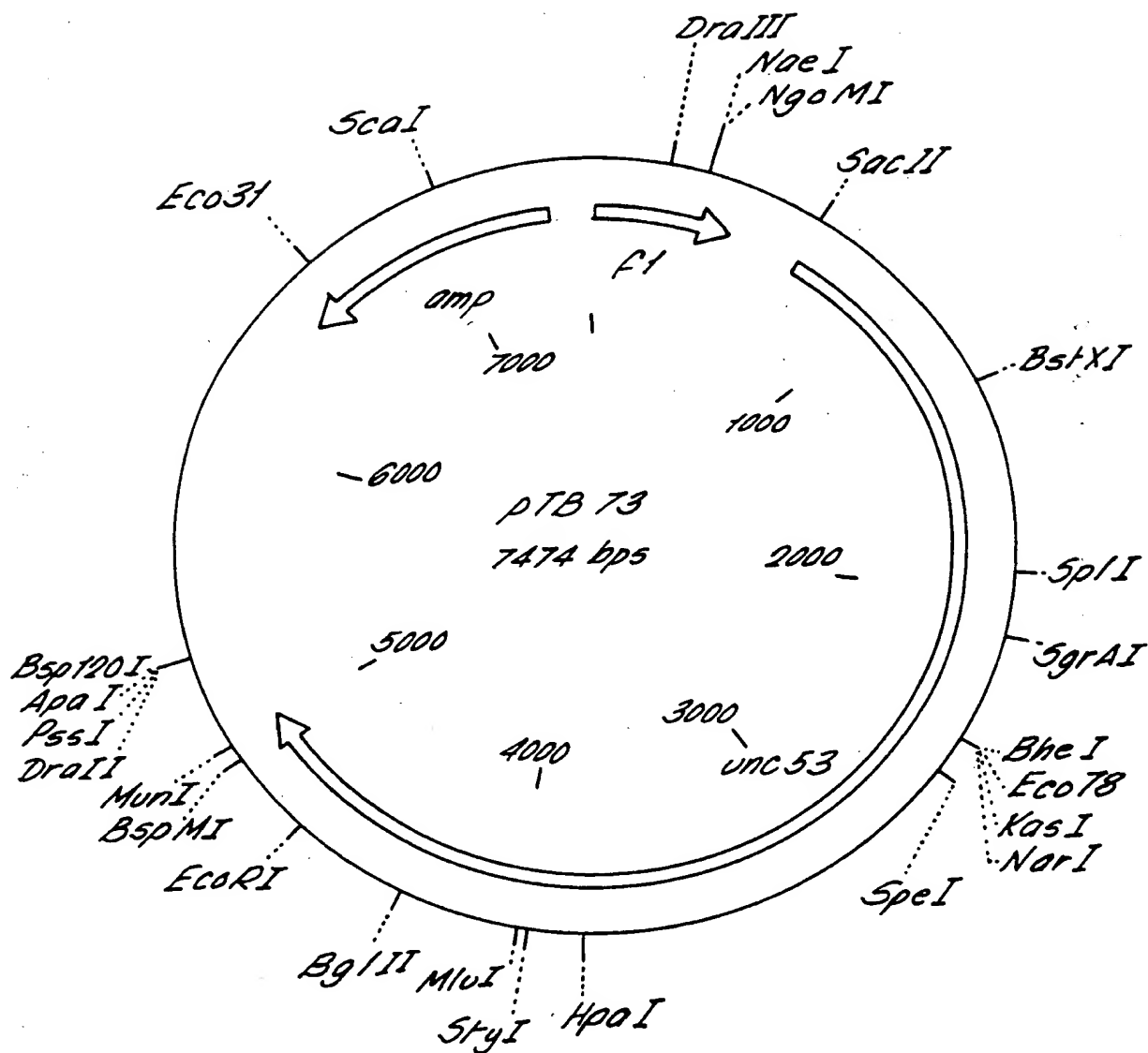
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## FIG. 26 CONTINUED

TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG	9180
GATCTTACCG CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGCACCCA ACTGATCTTC	9240
AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC	9300
AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCATA	9360
TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA	9420
GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC CTGACGTCGA	9480
CGGATCGGGA GATCTCCCGA TCCCCTATGG TCGACTCTCA GTACAATCTG CTCTGATGCC	9540
GCATAGTTAA GCCAGTATCT GCTCCCTGCT TGTGTGTTGG AGGTCGCTGA GTAGTGCGCG	9600
AGCAAAATTT AAGCTACAAC AAGGCAAGGC TTGACCGACA ATTGCATGAA GAATCTGCTT	9660
AGGGTTAGGC GTTTTGCGCT GCTTCGCGAT GTACGGGCCA GATATACGCG TTGACATTGA	9720
TTATTGACTA GTTATTAATA GTAATCAATT ACGGGGTCAT TAGTTCATAG CCCATATATG	9780
GAGTTCGCG TTACATAACT TACGGTAAAT GGCCCGCCTG GCTGACCGCC CAACGACCCC	9840
CGCCCATGTA CGTCAATAAT GACGTATGTT CCCATAGTAA CGCCAATAGG GACTTTCCAT	9900
TGACGTCAAT GGGTGGACTA TTTACGGTAA ACTGCCCCACT TGGCAGTACA TCAAGTGTAT	9960
CATATGCCAA GTACGCCCCC TATTGACGTC AATGACGGTA AATGGCCCCG CTGGCATTAT	10020
GCCCAGTACA TGACCTTATG GGACTTTCCT ACTTGGCAGT ACATCTACGT ATTAGTCATC	10080
GCTATTACCA TGGTGATGCG GTTTTGGCAG TACATCAATG GGCGTGGATA GCGGTTTGAC	10140
TCACGGGGAT TTCCAAGTCT CCACCCCAT TACGTCAATG GGAGTTTGTT TTGGCACCAA	10200
AATCAACGGG ACTTTCCAAA ATGTCGTAAC AACTCCGCCC CATTGACGCA AATGGGCGGT	10260
AGGCGTGTAC GGTGGGAGGT CTATATAAGC AGAGCTCTCT GGCTAACTAG AGAACCCACT	10320
GCTTACTGGC TTATCGAAAT TAATACGACT CACTATAGGG AGACCCAAGC TTGGTACCGA	10380
GCTCGGATCC ACTAGTAACG GCCGCCAGTG TGCTGGAATT CTGCAGATAT CCATCACACT	10440
GGC	10443

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FIG. 27.



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FIG. 28.

CTAAATTGTA AGCGTTAATA TTTTGTTAAA ATTCGCGTTA AATTTTGTGTT AAATCAGCTC	60
ATTTTTTAAC CAATAGGCCG AAATCGGCAA AATCCCTTAT AAATCAAAAG AATAGACCGA	120
GATAGGGTTG AGTGTGTGTC CAGTTTGGAA CAAGAGTCCA CTATTAAAGA ACGTGGACTC	180
CAACGTCAAA GGGCGAAAAA CCGTCTATCA GGGCGATGGC CCACTACGTG AACCATCACC	240
CTAATCAAGT TTTTTGGGGT CGAGGTGCCG TAAAGCACTA AATCGGAACC CTAAAGGGAG	300
CCCCGATTT AGAGCTTGAC GGGGAAAGCC GCGGAACGTG GCGAGAAAGG AAGGGAAGAA	360
AGCGAAAGGA GCGGGCGCTA GGGCGCTGGC AAGTGTAGCG GTCACGCTGC GCGTAACCAC	420
CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTCC CATTCGCCAT TCAGGCTGCG	480
CAACTGTTGG GAAGGGCGAT CGGTGCGGGC CTCTTCGCTA TTACGCCAGC TGGCGAAAGG	540
GGGATGTGCT GCAAGGCGAT TAAGTTGGGT AACGCCAGGG TTTTCCCAGT CACGACGTTG	600
TAAACGACG GCCAGTGAGC GCGCGTAATA CGACTCACTA TAGGGCGAAT TGGAGCTCCA	660
CCGCGGTTTC TAAATTACCC TCGCCACGTG TCGCCACGTC AGCAACCGCT TCAGCAACTA	720
ACCCAAATTC CAACTTTCCA CAAATGTCAA CATCCAGGCT TCAGACTCCA CAGTCAAGAA	780
TATCGAAAAT TGATTCATCA AAGATTGGTA TCAAGCCAAA GACGTCTGGA CTAAACCAC	840
CCTCATCATC AACCACTTCA TCAAATAATA CAAATTCATT CCGTCCGTCG AGCCGTTCTGA	900
GTGGCAATAA TAATGTTGGC TCGACGATAT CCACATCTGC GAAGAGCTTA GAATCATCAT	960
CAACGTACAG CTCTATTTTCG AATCTAAACC GACCTACCTC CCAACTCCAA AAACCTTCTA	1020
GACCACAAAC CCAGCTAGTT CGTGTGCTA CAACTACAAA AATCGGAAGC TCAAAGCTAG	1080
CCGCTCCGAA AGCCGTGAGC ACCCCAAAAC TTGCTTCTGT GAAGACTATT GGAGCAAAAC	1140
AAGAGCCCGA TAACAGCGGT GGTGGTGGTG GTGGAATGCT GAAATTAAAG TTATTCAGTA	1200
GCAAAAACCC ATCTTCCTCA TCGAATAGCC CACAACCTAC GAGAAAGGCG GCGGCGGTGC	1260
CTCAACAACA AACTTTGTCG AAAATCGCTG CCCAGTGAA AAGTGGCCTG AAGCCGCCGA	1320
CCAGTAAGCT GGGAAAGTGC ACGTCTATGT CGAAGCTTTG TACGCCAAAA GTTTCCTACC	1380
GTAAAACGGA CGCCCCAATC ATATCTCAAC AAGACTCGAA ACGATGCTCA AAGAGCAGTG	1440
AAGAAGAGTC CGGATACGCT GGATTCAACA GCACGTCGCC AACGTCATCA TCGACGGAAG	1500
GTTCCCTAAG CATGCATTCC ACATCTTCCA AGAGTTCAAC GTCAGACGAA AAGTCTCCGT	1560
CATCAGACGA TCTTACTCTT AACGCCTCCA TCGTGACAGC TATCAGACAG CCGATAGCCG	1620
CAACACCGGT TTCTCCAAAT ATTATCAACA AGCCTGTTGA GGAAAAACCA ACACTGGCAG	1680
TGAAAGGAGT GAAAAGCACA GCGAAAAAAG ATCCACCTCC AGCTGTTCCG CCACGTGACA	1740
CCCAGCCAAC AATCGGAGTT GTTAGTCCAA TTATGGCACA TAAGAAGTTG ACAAATGACC	1800
CCGTGATATC TGAAAAACCA GAACCTGAAA AGCTCCAATC AATGAGCATC GACACGACGG	1860

*FIG. 28 CONTINUED.**6/1/99*

ACGTTCCACC	GCTTCCACCT	CTAAAATCAG	TTGTTCCACT	TAAAATGACT	TCAATCCGAC	1920
AACCACCAAC	GTACGATGTT	CTTCTAAAAC	AAGGAAAAAT	CACATCGCCT	GTCAAGTCGT	1980
TTGGATATGA	GCAGTCGTCC	GCGTCTGAAG	ACTCCATTGT	GGCTCATGCG	TCGGCTCAGG	2040
TGACTCCGCC	GACAAAAACT	TCTGGTAATC	ATTCGCTGGA	GAGAAGGATG	GGAAAGAATA	2100
AGACATCAGA	ATCCAGCGGC	TACACCTCTG	ACGCCGGTGT	TGCGATGTGC	GCCAAAATGA	2160
GGGAGAAGCT	GAAAGAATAC	GATGACATGA	CTCGTCGAGC	ACAGAACGGC	TATCCTGACA	2220
ACTTCGAAGA	CAGTTCCTCC	TTGTCGTCTG	GAATATCCGA	TAACAACGAG	CTCGACGACA	2280
TATCCACGGA	CGATTTGTCC	GGAGTAGACA	TGGCAACAGT	CGCCTCCAAA	CATAGCGACT	2340
ATTCCCCTT	TGTTCCGCAT	CCCACGTCTT	CTTCCTCAA	GCCCCGAGTC	CCCAGTCGGT	2400
CCTCCACATC	AGTCGATTCT	CGATCTCGAG	CAGAACAGGA	GAATGTGTAC	AACTTCTGT	2460
CCCAGTGCCG	AACGAGCCAA	CGTGGCGCCG	CTGCCACCTC	AACCTTCGGA	CAACATTCGC	2520
TAAGATCCCC	GGGATACTCA	TCCTATTCTC	CACACTTATC	AGTGTGAGCT	GATAAGGACA	2580
CAATGTCTAT	GCACTCACAG	ACTAGTCGAC	GACCTTCTTC	ACAAAAACCA	AGCTATTCAG	2640
GCCAATTTCA	TTCACTTGAT	CGTAAATGCC	ACCTTCAAGA	GTTACATCC	ACCGAGCACA	2700
GAATGGCGGC	TCTCTTGAGC	CCGAGACGGG	TGCCGAACTC	GATGTCGAAA	TATGATTCTT	2760
CAGGATCCTA	CTCGGCGCGT	TCCCAGAGTG	GAAGCTCTAC	TGGTATCTAT	GGAGAGACGT	2820
TCCAAGTGCA	CAGACTATCC	GATGAAAAAT	CCCCCGCACA	TTCTGCCAAA	AGTGAGATGG	2880
GATCCCAACT	ATCACTGGCT	AGCACGACAG	CATATGGATC	TCTCAATGAG	AAGTACGAAC	2940
ATGCTATTCTG	GGACATGGCA	CGTGACTTGG	AGTGTTACAA	GAACACTGTC	GACTCACTAA	3000
CCAAGAAACA	GGAGAACTAT	GGAGCATTGT	TTGATCTTTT	TGAGCAAAAG	CTTAGAAAAC	3060
TCACTCAACA	CATTGATCGA	TCCAAGTTGA	AGCCTGAAGA	GGCAATACGA	TTCAGGCAGG	3120
ACATTGCTCA	TTTGAGGGAT	ATTAGCAATC	ATCTTGATC	CAACTCAGCT	CATGCTAACG	3180
AAGGCGCTGG	TGAGCTTCTT	CGTCAACCAT	CTCTGGAATC	AGTTGCATCC	CATCGATCAT	3240
CGATGTCATC	GTCGTCGAAA	AGCAGCAAGC	AGGAGAAGAT	CAGCTTGAGC	TCGTTTGCCA	3300
AGAACAAGAA	GAGCTGGATC	CGCTCCTCAC	TCTCCAAGTT	CACCAAGAAG	AAGAACAAGA	3360
ACTACGACGA	AGCACATATG	CCATCAATTT	CCGGATCTCA	AGGAACTCTT	GACAACATTG	3420
ATGTGATTGA	GTTGAAGCAA	GAGCTCAAAG	AACGCGATAG	TGCACTTTAC	GAAGTCCGCC	3480
TTGACAATCT	GGATCGTGCC	CGCGAAGTTG	ATGTTCTGAG	GGAGACAGTG	AACAAGTTGA	3540
AAACCGAGAA	CAAGCAATTA	AAGAAAGAAG	TGGACAAACT	CACCAACGGT	CCAGCCACTC	3600
GTGCTTCTTC	CCGCGCCTCA	ATTCCAGTTA	TCTACGACGA	TGAGCATGTC	TATGATGCAG	3660
CGTGTAGCAG	TACATCAGCT	AGTCAATCTT	CGAAACGATC	CTCTGGCTGC	AACTCAATCA	3720
AGGTTACTGT	AAACGTGGAC	ATCGCTGGAG	AAATCAGTTC	GATCGTTAAC	CCGGACAAAG	3780

*FIG. 28 CONTINUED.**62/99*

AGATAATCGT AGGATATCTT GCCATGTCAA CCAGTCAGTC ATGCTGGAAA GACATTGATG	3840
TTTCTATTCT AGGACTATTT GAAGTCTACC TATCCAGAAT TGATGTGGAG CATCAACTTG	3900
GAATCGATGC TCGTGATTCT ATCCTTGGCT ATCAAATTGG TGAACCTCGA CGCGTCATTG	3960
GAGACTCCAC AACCATGATA ACCAGCCATC CAACTGACAT TCTTACTTCC TCAACTACAA	4020
TCCGAATGTT CATGCACGGT GCCGCACAGA GTCGCGTAGA CAGTCTGGTC CTTGATATGC	4080
TTCTTCCAAA GCAAATGATT CTCCAACCTCG TCAAGTCAAT TTTGACAGAG AGACGTCTGG	4140
TGTTAGCTGG AGCAACTGGA ATTGGAAAGA GCAAACCTGGC GAAGACCCTG GCTGCTTATG	4200
TATCTATTCG AACAAATCAA TCCGAAGATA GTATTGTAA TATCAGCATT CCTGAAAACA	4260
ATAAAGAAGA ATTGCTTCAA GTGGAACGAC GCCTGGAAAA GATCTTGAGA AGCAAAGAAT	4320
CATGCATCGT AATTCTAGAT AATATCCCAA AGAATCGAAT TGCATTTGTT GTATCCGTTT	4380
TTGCAAATGT CCCACTTCAA AACACGAAG GTCCATTTGT AGTATGCACA GTCAACCGAT	4440
ATCAAATCCC TGAGCTTCAA ATTCACCACA ATTTCAAAT GTCAGTAATG TCGAATCGTC	4500
TCGAAGGATT CATCCTACGT TACCTCCGAC GACGGGCGGT AGAGGATGAG TATCGTCTAA	4560
CTGTACAGAT GCCATCAGAG CTCTTCAAAA TCATTGACTT CTTCCCAATA GCTCTTCAGG	4620
CCGTCAATAA TTTTATTGAG AAAACGAATT CTGTTGATGT GACAGTTGGT CCAAGAGCAT	4680
GCTTGAACCTG TCCTCTAACT GTCGATGGAT CCCGTGAATG GTTCATTCTGA TTGTGGAATG	4740
AGAACTTCAT TCCATATTTG GAACGTGTTG CTAGAGATGG CAAAAAAACC TTCGGTCGCT	4800
GCACTTCCTT CGAGGATCCC ACCGACATCG TCTCTAAAAA ATGGCCGTGG TTCGATGGTG	4860
AAAACCCGGA GAATGTGCTC AAACGTCTTC AACTCCAAGA CCTCGTCCCG TCACCTGCCA	4920
ACTCATCCCG ACAACACTTC AATCCCCTCG AGTCGTTGAT CCAATTGCAT GCTACCAAGC	4980
ATCAGACCAT CGACAACATT TGAACAGAAG ACTCTAATCT TCTCTCGCCT CTCCCCCGCT	5040
TTCTTATCT TCGTACCGGT ACCTGATGAT TCCCATTCTT CCCCCTTTT CCCCCAATTT	5100
CCCAGAACCT CCTGTTCCCT TTGTTCCCTAG TCCTCCCGGG TGCCGACGCC GAAGCGATTT	5160
AAAAACCTTT TTCTTTCCGA AACATTTCCC ATTGCTCATT AATAGTCAA TTGAATAAAC	5220
AGTGTATGTA CTAAAAAAA AAAAAAAAAA AACTCGAGGG GGGGCCCGGT ACCCAGCTTT	5280
TGTTCCCTTT AGTGAGGGTT AATTGCGCGC TTGGCGTAAT CATGGTCATA GCTGTTTCCT	5340
GTGTGAAATT GTTATCCGCT CACAATTCCA CACAACATAC GAGCCGGAAG CATAAAGTGT	5400
AAAGCCTGGG GTGCCAATG AGTGAGCTAA CTCACATTAA TTGCGTTGCG CTCACTGCCC	5460
GCTTTCCAGT CGGGAAACCT GTCGTGCCAG CTGCATTAAT GAATCGGCCA ACGCGCGGGG	5520
AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG	5580
GTCGTTCCGC TGCGGCGAGC GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA	5640
GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC	5700

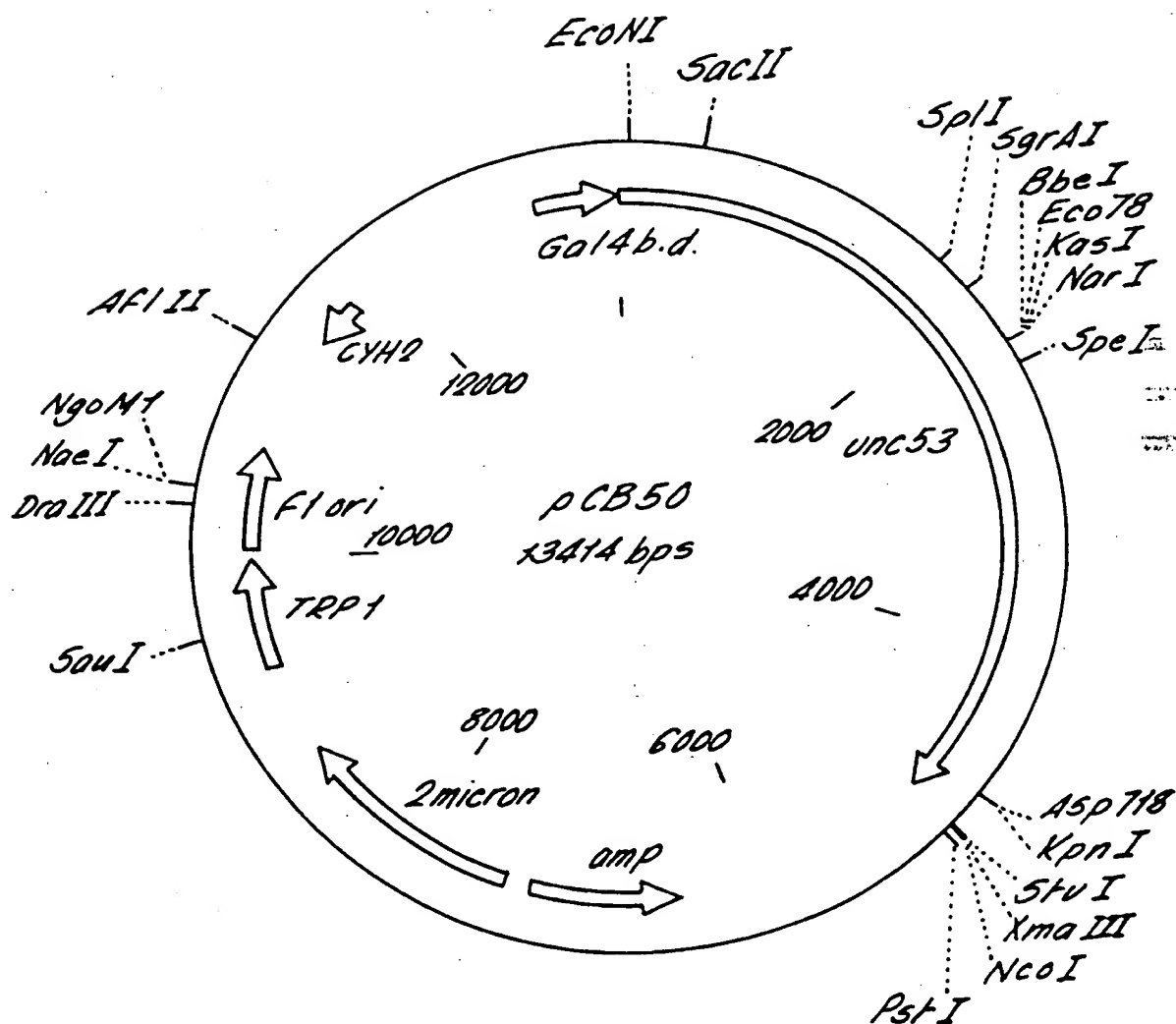
FIG. 28 CONTINUED.

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CGTAAAAAGG CCGCGTTGCT GCGGTTTTTC CATAGGCTCC GCCCCCTGA CGAGCATCAC	5760
AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG	5820
TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC	5880
CTGTCCGCTT TTCTCCCTTC GGGGAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT	5940
CTCAGTTCGG TGTAAGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCCTTCAG	6000
CCCGACCGCT GCGCCTTATC CGGTAACATC CGTCTTGAGT CCAACCCGGT AAGACACGAC	6060
TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT	6120
GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT	6180
ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC	6240
AAACAAACCA CCGCTGGTAG CCGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA	6300
AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC	6360
GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC	6420
CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT	6480
GACAGTTACC AATGCTTAAT CAGTGAGGCA CCTATCTCAG CGATCTGTCT ATTTCTGTTCA	6540
TCCATAGTTG CCTGACTCCC CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT	6600
GGCCCCAGTG CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA	6660
ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC CTGCAACTTT ATCCGCCTCC	6720
ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT TAATAGTTTG	6780
CGCAACGTTG TTGCCATTGC TACAGGCATC GTGGTGTCAC GCTCGTCGTT TGGTATGGCT	6840
TCATTAGCT CCGGTTCCCA ACGATCAAGG CGAGTTACAT GATCCCCCAT GTTGTCAGAA	6900
AAAGCGGTTA GTCCTTCGG TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA	6960
TCATCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG TCATGCCATC CGTAAGATGC	7020
TTTTCTGTGA CTGGTGAGTA CTCAACCAAG TCATTCTGAG AATAGTGTAT GCGGCGACCG	7080
AGTTGCTCTT GCGCGGCGTC AATACGGGAT AATACCGCGC CACATAGCAG AACTTTAAAA	7140
GTGCTCATCA TTGGAAAACG TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG	7200
AGATCCAGTT CGATGTAACC CACTCGTGCA CCCAAGTATC CTTGAGCATC TTTTACTTTC	7260
ACCAGCGTTT CTGGGTGAGC AAAAACAGGA AGGCAAAATG CCGCAAAAAA GGAATAAGG	7320
GCGACACGGA AATGTTGAAT ACTCATACTC TTCCTTTTTT AATATTATTG AAGCATTTAT	7380
CAGGGTTATT GTCTCATGAG CGGATACATA TTTGAATGTA TTTAGAAAAA TAAACAAATA	7440
GGGGTTCCGC GCACATTTCC CCGAAAAGTG CCAC	7474

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FIG. 29.





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FIG. 30.

TATGACGACG TCAAATGTAG AATTGATACC ATTCTACACG GATTGGGCCA ATCGGCACCT	60
TTCGAAGGGC AGCTTATCAA AGTCGATTAG GGATATTTCC AATGATTTTC GCGACTATCG	120
ACTGGTTTCT CAGCTTATTA ATGTGATCGT TCCGATCAAC GAATTCTCGC CTGCATTAC	180
GAAACGTTTG GCAAAAATCA CATCGAACCT GGATGGCCTC GAAACGTGTC TCGACTACCT	240
GAAAAATCTG GGTCTCGACT GCTCGAACT CACCAAAACC GATATCGACA GCGGAACTT	300
GGGTGCAGTT CTCCAGCTGC TCTTCCTGCT CTCCACCTAC AAGCAGAAGC TTCGGCAACT	360
GAAAAAAGAT CAGAAGAAAT TGGAGCAACT ACCCACATCC ATTATGCCAC CCGCGGTTTC	420
TAAATTACCC TCGCCACGTG TCGCCACGTC AGCAACCGCT TCAGCAACTA ACCCAAATTC	480
CAACTTTCCA CAAATGTCAA CATCCAGGCT TCAGACTCCA CAGTCAAGAA TATCGAAAAAT	540
TGATTTCATCA AAGATTGGTA TCAAGCCAAA GACGTCTGGA CTTAAACCAC CCTCATCATC	600
AACCACTTCA TCAAATAATA CAAATTCATT CCGTCCGTCG AGCCGTTCGA GTGGCAATAA	660
TAATGTTGGC TCGACGATAT CCACATCTGC GAAGAGCTTA GAATCATCAT CAACGTACAG	720
CTCTATTTCTG AATCTAAACC GACCTACCTC CCAACTCCAA AAACCTTCTA GACCACAAAC	780
CCAGCTAGTT CGTGTGCTA CAACTACAAA AATCGGAAGC TCAAAGCTAG CCGCTCCGAA	840
AGCCGTGAGC ACCCCAAAAC TTGCTTCTGT GAAGACTATT GGAGCAAAAC AAGAGCCCGA	900
TAACAGCGGT GGTGGTGGTG GTGGAATGCT GAAATTAAAG TTATTCAGTA GCAAAAACCC	960
ATCTTCCTCA TCGAATAGCC CACAACCTAC GAGAAAGGCG GCGGCGGTGC CTCAACAACA	1020
AACTTTGTCTG AAAATCGCTG CCCCAGTGAA AAGTGGCCTG AAGCCGCCGA CCAGTAAGCT	1080
GGGAAGTGCC ACGTCTATGT CGAAGCTTTG TACGCCAAAA GTTTCCTACC GTAAACGGA	1140
CGCCCCAATC ATATCTCAAC AAGACTCGAA ACGATGCTCA AAGAGCAGTG AAGAAGAGTC	1200
CGGATACGCT GGATTCAACA GCACGTCGCC AACGTCATCA TCGACGGAAG GTTCCCTAAG	1260

FIG. 30 CONTINUED.

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CATGCATTCC	ACATCTTCCA	AGAGTTCAAC	GTCAGACGAA	AAGTCTCCGT	CATCAGACGA	1320
TCTTACTCTT	AACGCCTCCA	TCGTGACAGC	TATCAGACAG	CCGATAGCCG	CAACACCCGT	1380
TTCTCCAAAT	ATTATCAACA	AGCCTGTTGA	GGAAAAACCA	ACACTGGCAG	TGAAAGGAGT	1440
GAAAAGCACA	GCGAAAAAAG	ATCCACCTCC	AGCTGTTCCG	CCACGTGACA	CCCAGCCAAC	1500
AATCGGAGTT	GTTAGTCCAA	TTATGGCACA	TAAGAAGTTG	ACAAATGACC	CCGTGATATC	1560
TGAAAAACCA	GAACCTGAAA	AGCTCCAATC	AATGAGCATC	GACACGACGG	ACGTTCCACC	1620
GCTTCCACCT	CTAAATCAG	TTGTTCCACT	TAAAATGACT	TCAATCCGAC	AACCACCAAC	1680
GTACGATGTT	CTTCTAAAAC	AAGGAAAAAT	CACATCGCCT	GTCAAGTCGT	TTGGATATGA	1740
GCAGTCGTCC	GCGTCTGAAG	ACTCCATTGT	GGCTCATGCG	TCGGCTCAGG	TGACTCCGCC	1800
GACAAAAACT	TCTGGTAATC	ATTCGCTGGA	GAGAAGGATG	GGAAAGAATA	AGACATCAGA	1860
ATCCAGCGGC	TACACCTCTG	ACGCCGGTGT	TGCGATGTGC	GCCAAAATGA	GGGAGAAGCT	1920
GAAAGAATAC	GATGACATGA	CTCGTCGAGC	ACAGAACGGC	TATCCTGACA	ACTTCGAAGA	1980
CAGTTCCTCC	TTGTCGTCTG	GAATATCCGA	TAACAACGAG	CTCGACGACA	TATCCACGGA	2040
CGATTTGTCC	GGAGTAGACA	TGGCAACAGT	CGCCTCCAAA	CATAGCGACT	ATTCCCCTT	2100
TGTTGCGCAT	CCCACGTCTT	CTTCCTCAA	GCCCCGAGTC	CCCAGTCGGT	CCTCCACATC	2160
AGTCGATTCT	CGATCTCGAG	CAGAACAGGA	GAATGTGTAC	AACTTCTGT	CCCAGTGCCG	2220
AACGAGCCAA	CGTGGCGCCG	CTGCCACCTC	AACCTTCGGA	CAACATTCGC	TAAGATCCCC	2280
GGGATACTCA	TCCTATTCTC	CACACTTATC	AGTGTGAGCT	GATAAGGACA	CAATGTCTAT	2340
GCACTCACAG	ACTAGTCGAC	GACCTTCTTC	ACAAAAACCA	AGCTATTCAG	GCCAATTTCA	2400
TTCACTTGAT	CGTAAATGCC	ACCTTCAAGA	GTTTACATCC	ACCGAGCACA	GAATGGCGGC	2460
TCTCTTGAGC	CCGAGACGGG	TGCCGAACTC	GATGTCGAAA	TATGATTCTT	CAGGATCCTA	2520
CTCGGCGCGT	TCCCGAGGTG	GAAGCTCTAC	TGGTATCTAT	GGAGAGACGT	TCCAAC TGCA	2580
CAGACTATCC	GATGAAAAAT	CCCCCGCACA	TTCTGCCAAA	AGTGAGATGG	GATCCCAACT	2640
ATCACTGGCT	AGCACGACAG	CATATGGATC	TCTCAATGAG	AAGTACGAAC	ATGCTATTCTG	2700
GGACATGGCA	CGTGACTTGG	AGTGTTACAA	GAACACTGTC	GACTCACTAA	CCAAGAAACA	2760
GGAGAACTAT	GGAGCATTGT	TTGATCTTTT	TGAGCRAAAG	CTTAGAAAAC	TCACTCAACA	2820
CATTGATCGA	TCCAACCTGA	AGCCTGAAGA	GGCAATACGA	TTCAGGCAGG	ACATTGCTCA	2880
TTTGAGGGAT	ATTAGCAATC	ATCTTGCATC	CAACTCAGCT	CATGCTAACG	AAGGCGCTGG	2940
TGAGCTTCTT	CGTCAACCAT	CTCTGGAATC	AGTTGCATCC	CATCGATCAT	CGATGTCATC	3000
GTCGTCGAAA	AGCAGCAAGC	AGGAGAAGAT	CAGCTTGAGC	TCGTTTGGCA	AGAACAAGAA	3060
GAGCTGGATC	CGCTCCTCAC	TCTCCAAGTT	CACCAAGAAG	AAGAACAAGA	ACTACGACGA	3120
AGCACATATG	CCATCAATTT	CCGGATCTCA	AGGAACTCTT	GACAACATTG	ATGTGATTGA	3180

FIG. 30 CONTINUED.

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GTTGAAGCAA GAGCTCAAAG AACGCGATAG TGCACCTTAC GAAGTCCGCC TTGACAATCT	3240
GGATCGTGCC CGCGAAGTTG ATGTTCTGAG GGAGACAGTG AACAAAGTTGA AAACCGAGAA	3300
CAAGCAATTA AAGAAAGAAG TGGACAAACT CACCAACGGT CCAGCCACTC GTGCTTCTTC	3360
CCGCGCCTCA ATTCCAGTTA TCTACGACGA TGAGCATGTC TATGATGCAG CGTGTAGCAG	3420
TACATCAGCT AGTCAATCTT CGAAACGATC CTCTGGCTGC AACTCAATCA AGGTTACTGT	3480
AAACGTGGAC ATCGCTGGAG AAATCAGTTC GATCGTTAAC CCGGACAAAG AGATAATCGT	3540
AGGATATCTT GCCATGTCAA CCAGTCAGTC ATGCTGGAAA GACATTGATG TTTCTATTCT	3600
AGGACTATTT GAAGTCTACC TATCCAGAAT TGATGTGGAG CATCAACTTG GAATCGATGC	3660
TCGTGATTCT ATCCTTGGCT ATCAAATTGG TGAACCTCGA CGCGTCATTG GAGACTCCAC	3720
AACCATGATA ACCAGCCATC CAACTGACAT TCTTACTTCC TCAACTACAA TCCGAATGTT	3780
CATGCACGGT GCCGCACAGA GTCGCGTAGA CAGTCTGGTC CTTGATATGC TTCTTCCAAA	3840
GCAAATGATT CTCCAACCTG TCAAGTCAAT TTTGACAGAG AGACGTCTGG TGTTAGCTGG	3900
AGCAACTGGA ATTGGAAAGA GCAAACCTGG GAAGACCCTG GCTGCTTATG TATCTATTCTG	3960
AACAAATCAA TCCGAAGATA GTATTGTTAA TATCAGCATT CCTGAAAACA ATAAAGAAGA	4020
ATTGCTTCAA GTGGAACGAC GCCTGGAAAA GATCTTGAGA AGCAAAGAAT CATGCATCGT	4080
AATTCTAGAT AATATCCCAA AGAATCGAAT TGCATTTGTT GTATCCGTTT TTGCAAATGT	4140
CCCACTTCAA AACAAACGAAG GTCCATTTGT AGTATGCACA GTCAACCGAT ATCAAATCCC	4200
TGAGCTTCAA ATTCACCACA ATTTCAAAT GTCAGTAATG TCGAATCGTC TCGAAGGATT	4260
CATCCTACGT TACCTCCGAC GACGGGCGGT AGAGGATGAG TATCGTCTAA CTGTACAGAT	4320
GCCATCAGAG CTCTTCAAAA TCATTGACTT CTTCCCAATA GCTCTTCAGG CCGTCAATAA	4380
TTTTATTGAG AAAACGAATT CTGTTGATGT GACAGTTGGT CCAAGAGCAT GCTTGAACCTG	4440
TCTCTAACT GTCGATGGAT CCCGTGAATG GTTCATTCTGA TTGTGGAATG AGAACTTCAT	4500
TCCATATTTG GAACGTGTTG CTAGAGATGG CAAAAAACC TTCGGTCGCT GCACTTCCTT	4560
CGAGGATCCC ACCGACATCG TCTCTAAAAA ATGGCCGTGG TTCGATGGTG AAAACCCGGA	4620
GAATGTGCTC AAACGTCTTC AACTCCAAGA CCTCGTCCCG TCACCTGCCA ACTCATCCCG	4680
ACAACACTTC AATCCCCTCG AGTCGTTGAT CCAATTGCAT GCTACCAAGC ATCAGACCAT	4740
CGACAACATT TGAACAGAAG ACTCTAATCT TCTCTCGCCT CTCCCCCGCT TTCCTTATCT	4800
TCGTACCGGT ACCTGATGAT TCCCCATTTT CCCCCTTTTC CCCCCAATTT CCCAGAACCT	4860
CCTGTTCCCT TTGTTCTAG TCCTCCCGGG TGCCGACGCC GAAGCGATTT AAAAACCTTT	4920
TTCTTTCCGA AACATTTCCC ATTGCTCATT AATAGTCAAA TTGAATAAAC AGTGTATGTA	4980
CTTAAAAAAA AAAAAAAAAA AAAAAAAAAA GGCCTATGCG GCCGGGCCAT GGAGGCCGAA	5040
TTCCCGGGGA TCCGTCGACC TGCAGCCAAG CTAATTCCGG GCGAATTTCT TATGATTTAT	5100

*FIG. 30 CONTINUED.* 68/99

GATTTTTATT ATTAATAAG TTATAAAAAA AATAAGTGTA TACAAATTTT AAAGTGACTC	5160
TTAGGTTTTA AAACGAAAAT TCTTGTTCTT GAGTAACTCT TTCCTGTAGG TCAGGTTGCT	5220
TTCTCAGGTA TAGCATGAGG TCGCTCTTAT TGACCACACC TCTACCGGCA TGCAAGCTTG	5280
GCGTAATCAT GGTTCATAGCT GTTTCCTGTG TGAAATTGTT ATCCGCTCAC AATTCCACAC	5340
AACATACGAG CCGGAAGCAT AAAGTGTAAG GCCTGGGGTG CCTAATGAGT GAGGTAAGTC	5400
ACATTAATTG CGTTGCGCTC ACTGCCCGCT TTCCAGTCGG GAAACCTGTC GTGCCAGCTG	5460
GATTAATGAA TCGGCCAACG CGCGGGGAGA GCGGGTTTGC GTATTGGGCG CTCTTCCGCT	5520
TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC GCGGAGCGGT ATCAGCTCAC	5580
TCAAAGGCGG TAATACGGTT ATCCACAGAA TCAGGGGATA ACGCAGGAAA GAACATGTGA	5640
GCAAAAGGCC AGCAAAAGGC CAGGAACCGT AAAAAGGCCG CGTTGCTGGC GTTTTCCAT	5700
AGGCTCCGCC CCCCTGACGA GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC	5760
CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA GCTCCCTCGT GCGCTCTCCT	5820
GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG	5880
CTTTCTCATA GCTCACGCTG TAGGTATCTC AGTTCGGTGT AGGTCGTTTC CTCCAAGCTG	5940
GGCTGTGTGC ACGAACCCCC CGTTCAGCCC GACCGCTGCG CTTATCCGG TAACTATCGT	6000
CTTGAGTCCA ACCCGGTAAG ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG	6060
ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC	6120
GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC TGAAGCCAGT TACCTTCGGA	6180
AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT	6240
GTTTGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT	6300
TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT AAGGGATTTT GGTATGAGA	6360
TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC	6420
TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT	6480
ATCTCAGCGA TCTGTCTATT TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA	6540
ACTACGATAC GGGAGGGCTT ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA	6600
CGCTCACC GG CTCCAGATTT ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA	6660
AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA	6720
GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG	6780
GTGTCACGCT CGTCGTTTGG TATGGCTTCA TTCAGCTCCG GTTCCCAACG ATCAAGGCGA	6840
GTTACATGAT CCCCCATGTT GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT	6900
GTCAGAAGTA AGTTGGCCGC AGTGTTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT	6960
CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA	7020

*FIG. 30 CONTINUED.* *69/99*

TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC CGGCGTCAAT ACGGGATAAT	7080
ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG CTCATCATTG GAAAACGTTT TTCGGGGCGA	7140
AAACTCTCAA GGATCTTACC GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGCACCC	7200
AACTGATCTT CAGCATCTTT TACTTTTACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG	7260
CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC	7320
CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT	7380
GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA CATTTCCTCCG AAAAGTGCCA	7440
CCTGAACGAA GCATCTGTGC TTCATTTTGT AGAACAAAAA TGCAACGCGA GAGCGCTAAT	7500
TTTTCAAACA AAGAATCTGA GCTGCATTTT TACAGAACAG AAATGCAACG CGAAAGCGCT	7560
ATTTTACCAA CGAAGAATCT GTGCTTCATT TTTGTAAAC AAAAATGCAA CGCGAGAGCG	7620
CTAATTTTTT AAACAAAGAA TCTGAGCTGC ATTTTTTACAG AACAGAAATG CAACGCGAGA	7680
GCGCTATTTT ACCAACAAAG AATCTATACT TCTTTTTTGT TCTACAAAAA TGCATCCCGA	7740
GAGCGCTATT TTTCTAACAA AGCATCTTAG ATTACTTTTT TTCTCCTTTG TGCGCTCTAT	7800
AATGCAGTCT CTTGATAACT TTTTGCCTG TAGGTCCGTT AAGGTTAGAA GAAGGCTACT	7860
TTGGTGTCTA TTTTCTCTTC CATAAAAAAA GCCTGACTCC ACTTCCCGCG TTTACTGATT	7920
ACTAGCGAAG CTGCGGGTGC ATTTTTTCAA GATAAAGGCA TCCCCGATTA TATTCTATAC	7980
CGATGTGGAT TGCGCATACT TTGTGAACAG AAAGTGATAG CGTTGATGAT TCTTCATTGG	8040
TCAGAAAATT ATGAACGGTT TCTTCTATTT TGTCTCTATA TACTACGTAT AGGAAATGTT	8100
TACATTTTCG TATTGTTTTT GATTCACTCT ATGAATAGTT CTTACTACAA TTTTTTTGTC	8160
TAAAGAGTAA TACTAGAGAT AAACATAAAA AATGTAGAGG TCGAGTTTAG ATGCAAGTTC	8220
AAGGAGCGAA AGGTGGATGG GTAGGTTATA TAGGGATATA GCACAGAGAT ATATAGCAAA	8280
GAGATACTTT TGAGCAATGT TTGTGGAAGC GGTATTCGCA ATATTTTAGT AGCTCGTTAC	8340
AGTCCGGTGC GTTTTTGGTT TTTTGAAAGT GCGTCTTCAG AGCGCTTTTG GTTTTCAAAA	8400
GCGCTCTGAA GTTCTATAC TTTCTAGAGA ATAGGAACTT CGGAATAGGA ACTTCAAAGC	8460
GTTTCCGAAA ACGAGCGCTT CCGAAAATGC AACGCGAGCT GCGCACATAC AGCTCACTGT	8520
TCACGTCGCA CCTATATCTG CGTGTTGCCT GTATATATAT ATACATGAGA AGAACGGCAT	8580
AGTGCGTGTT TATGCTTAAA TGCGTACTTA TATGCGTCTA TTTATGTAGG ATGAAAGGTA	8640
GTCTAGTACC TCCTGTGATA TTATCCCATT CCATGCGGGG TATCGTATGC TTCCTTCAGC	8700
ACTACCCTTT AGCTGTTCTA TATGCTGCCA CTCCTCAATT GGATTAGTCT CATCCTTCAA	8760
TGCTATCATT TCCTTTGATA TTGGATCATA TTAAGAAACC ATTATTATCA TGACATTAAAC	8820
CTATAAAAAT AGGCGTATCA CGAGGCCCTT TCGTCTCGCG CGTTTCGGTG ATGACGGTGA	8880
AAACCTCTGA CACATGCAGC TCCCGGAGAC GGTCACAGCT TGTCTGTAAG CGGATGCCGG	8940

*FIG. 30 CONTINUED**70/99*

GAGCAGACAA	GCCCCTCAGG	GCGCGTCAGC	GGGTGTTGGC	GGGTGTCGGG	GCTGGCTTAA	9000
CTATGCGGCA	TCAGAGCAGA	TTGTACTGAG	AGTGCACCAT	AGATCAACGA	CATTACTATA	9060
TATATAATAT	AGGAAGCATT	TAATAGACAG	CATCGTAATA	TATGTGTACT	TTGCAGTTAT	9120
GACGCCAGAT	GGCAGTAGTG	GAAGATATTC	TTTATTGAAA	AATAGCTTGT	CACCTTACGT	9180
ACAATCTTGA	TCCGGAGCTT	TTCTTTTTTT	GCCGATTAAG	AATTAATTCG	GTCGAAAAAA	9240
GAAAAGGAGA	GGGCCAAGAG	GGAGGGCATT	GGTGA CTATT	GAGCACGTGA	GTATACGTGA	9300
TTAAGCACAC	AAAGGCAGCT	TGGAGTATGT	CTGTTATTAA	TTTCACAGGT	AGTTCTGGTC	9360
CATTGGTGAA	AGTTTGCGGC	TTGCAGAGCA	CAGAGGCCGC	AGAATGTGCT	CTAGATTCCG	9420
ATGCTGACTT	GCTGGGTATT	ATATGTGTGC	CCAATAGAAA	GAGAACAATT	GACCCGGTTA	9480
TTGCAAGGAA	AATTTCAAGT	CTTGTA AAAAG	CATATAAAAA	TAGTTCAGGC	ACTCCGAAAT	9540
ACTTGGTTGG	CGTGTTTCGT	AATCAACCTA	AGGAGGATGT	TTTGGCTCTG	GTCAATGATT	9600
ACGGCATTGA	TATCGTCCAA	CTGCATGGAG	ATGAGTCGTG	GCAAGAATAC	CAAGAGTTCC	9660
TCGGTTTGCC	AGTTATTAAA	AGACTCGTAT	TTCCAAAAGA	CTGCAACATA	CTACTCAGTG	9720
CAGCTTCACA	GAAACCTCAT	TCGTTTATTC	CCTTGTTTGA	TTCAGAAGCA	GGTGGGACAG	9780
GTGAAC TTTT	GGATTGGAAC	TCGATT TCTG	ACTGGGTTGG	AAGGCAAGAG	AGCCCCGAAA	9840
GCTTACATTT	TATGTTAGCT	GGTGGACTGA	CGCCAGAAAA	TGTTGGTGAT	GCGCTTAGAT	9900
TAAATGGCGT	TATTGGTGTT	GATGTAAGCG	GAGGTGTGGA	GACAAATGGT	GTAAAAGACT	9960
CTAACAAAAT	AGCAAATTC	GTCAAAAATG	CTAAGAAATA	GGTTATTACT	GAGTAGTATT	10020
TATTTAAGTA	TTGTTTGTGC	ACTTGCCGAT	CTATGCGGTG	TGAAATACCG	CACAGATGCG	10080
TAAGGAGAAA	ATACCGCATC	AGGAAATTGT	AAACGTTAAT	ATTTTGTTAA	AATTCGCGTT	10140
AAATTTTGT	TAAATCAGCT	CATTTTTTAA	CCAATAGGCC	GAAATCGGCA	AAATCCCTTA	10200
TAAATCAAAA	GAATAGACCG	AGATAGGGTT	GAGTGTGTG	CCAGTTTGGA	ACAAGAGTCC	10260
ACTATTAAAG	AACGTGGACT	CCAACGTCAA	AGGGCGAAAA	ACCGTCTATC	AGGGCGATGG	10320
CCCACTACGT	GAACCATCAC	CCTAATCAAG	TTTTTTGGGG	TCGAGGTGCC	GTAAAGCACT	10380
AAATCGGAAC	CCTAAAGGGA	GCCCCCGATT	TAGAGCTTGA	CGGGGAAAGC	CGGCGAACGT	10440
GGCGAGAAAG	GAAGGGAAGA	AAGCGAAAGG	AGCGGGCGCT	AGGGCGCTGG	CAAGTGTAGC	10500
GGTCACGCTG	CGCGTAACCA	CCACACCCGC	CGCGCTTAAT	GCGCCGCTAC	AGGGCGCGTC	10560
GCGCCATTTC	CCATTCAGGC	TGCGCAACTG	TTGGGAAGGG	CGATCGGTGC	GGGCTCTTC	10620
GCTATTACGC	CAGCTGGCGA	AAGGGGGATG	TGCTGCAAGG	CGATTAAAGT	GGGTAACGCC	10680
AGGGTTTTCC	CAGTCACGAC	GTTGTAAAAC	GACGGCCAGT	CGTCCAAGCT	TTGCGGAGCT	10740
CGAGATCCCG	AGCTTTGCAA	ATTAAAGCCT	TCGAGCGTCC	CAAAACCTTC	TCAAGCAAGG	10800
TTTTCAGTAT	AATGTTACAT	GCGTACACGC	GTCTGTACAG	AAAAAAAAGA	AAAATTTGAA	10860

FIG. 30 CONTINUED.

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ATATAAATAA	CGTTCTTAAT	ACTAACATAA	CTATAAAAAA	ATAAATAGGG	ACCTAGACTT	10920
CAGGTTGTCT	AACTCCTTCC	TTTTCGGTTA	GAGCGGATGT	GGGGGGAGGG	CGTGAATGTA	10980
AGCGTGACAT	AACTAATTAC	ATGATATCGA	CAAAGGAAAA	GGGGCCTGTT	TACTCACAGG	11040
CTTTTTTCAA	GTAGGTAATT	AAGTCGTTTC	TGTCTTTTTT	CTTCTTCAAC	CCACCAAAGG	11100
CCATCTTGGT	ACTTTTTTTT	TTTTTTTTTT	TTTTTTTTTT	TTTTTTTTTT	TTTTTTTTTT	11160
TTTTTTTTTT	TTTTTTTTTT	TTTTTTTTTT	TTTTTTTTTT	TTTTTTTCATA	GAAATAATAC	11220
AGAAGTAGAT	GTTGAATTAG	ATTAAACTGA	AGATATATAA	TTTATTGGAA	AATACATAGA	11280
GCTTTTTGTT	GATGCGCTTA	AGCGATCAAT	TCAACAACAC	CACCAGCAGC	TCTGATTTTT	11340
TCTTCAGCCA	ACTTGAGAC	GAATCTAGCT	TTGACGATAA	CTGGAACATT	TGGGATTCTA	11400
CCCTTACCCA	AGATCTTACC	GTAACCGGCT	GCCAAAGTGT	CAATAACTGG	AGCAGTTTCC	11460
TTAGAAGCAG	ATTTCAAGTA	TTGGTCTCTC	TTGTCTCTCG	GGATCAATGT	CCACAATTTG	11520
TCCAAGTTCA	AGACTGGCTT	CCAGAAATGA	GCTTGTTGCT	TGTGGAAGTA	TCTCATACCA	11580
ANCCTTACCG	AAATAACCTG	GATGGTATTT	ATCCATGTTA	ATTCTGTGGT	GATGTTGACC	11640
ACCGGCCATA	CCTCTACCAC	CGGGGTGCTT	TCTGTGCTTA	CCGATACGAC	CTTTACCGGC	11700
TGAGACGTGA	CCTCTGTGCT	TTCTAGTCTT	AGTGAATCTG	GAAGGCATTG	TTGATTAGTT	11760
GGATGATTGT	TCTGGGATTT	AATGCAAAAA	AATCACTAAG	AAGGAAAAAA	ATCAACGGAG	11820
AAAGCAAACG	CCATCTTAAA	TATACGGGAT	ACAGATGAAA	GGTTTGAACC	TATCTGGGAA	11880
AATACGCATT	AAACAAGCGA	AAAACCTGCGA	GGAAAAATTGT	TTGCGTCTCT	GCGGGCTATT	11940
CACGCGCCAG	AGGAAAATAG	GAAAAATAAC	AGGGCATTAG	AAAAATAATT	TTGATTTTGG	12000
TAATGTGTGG	GTCCCTGGTG	TACAGATGTT	ACATTGGTTA	CAGTACTCTT	GTTTTTGCTG	12060
TGTTTTTCGA	TGAATCTCCA	AAATGGTTGT	TAGCACATGG	AAGAGTCACC	GATGCTAAGT	12120
TATCTCTATG	TAAGCTACGT	GGCGTGAAGT	TTGATGAAGC	CGCACAAAGAG	ATACAGGATT	12180
GGCAACTGCA	AATAGAATCT	GGGGATCTAG	ATATCCTTTT	GTTGTTTCCG	GGTGTAACAT	12240
ATGGACTTCC	TCTTTTCTGG	CAACCAAACC	CATACATCGG	GATTCCTATA	ATACCTTCGT	12300
TGGTCTCCCT	AACATGTAGG	TGGCGGAGGG	GAGATATACA	ATAGAACAGA	TACCAGACAA	12360
GACATAATGG	GCTAAACAAG	ACTACACCAA	TTACACTGCC	TCATTGATGG	TGGTACATAA	12420
CGAACTAATA	CTGTAGCCCT	AGACTTGATA	GCCATCATCA	TATCGAAGTT	TCCTACCCT	12480
TTTTCCATTT	GCCATCTATT	GAAGTAATAA	TAGGCGCATG	CAACTTCTTT	TCTTTTTTTT	12540
TCTTTTCTCT	CTCCCCCGTT	GTTGTCTCAC	CATATCCGCA	ATGACAAAAA	AAATGATGGA	12600
AGACACTAAA	GGAAAAAATT	AACGACAAAG	ACAGCACCAA	CAGATGTCGT	TGTTCCAGAG	12660
CTGATGAGGG	GTATCTTCGA	ACACACGAAA	CTTTTTCCTT	CCTTCATTCA	CGCACACTAC	12720
TCTCTAATGA	GCAACGGTAT	ACGGCCTTCC	TTCCAGTTAC	TTGAATTTGA	AATAAAAAAA	12780

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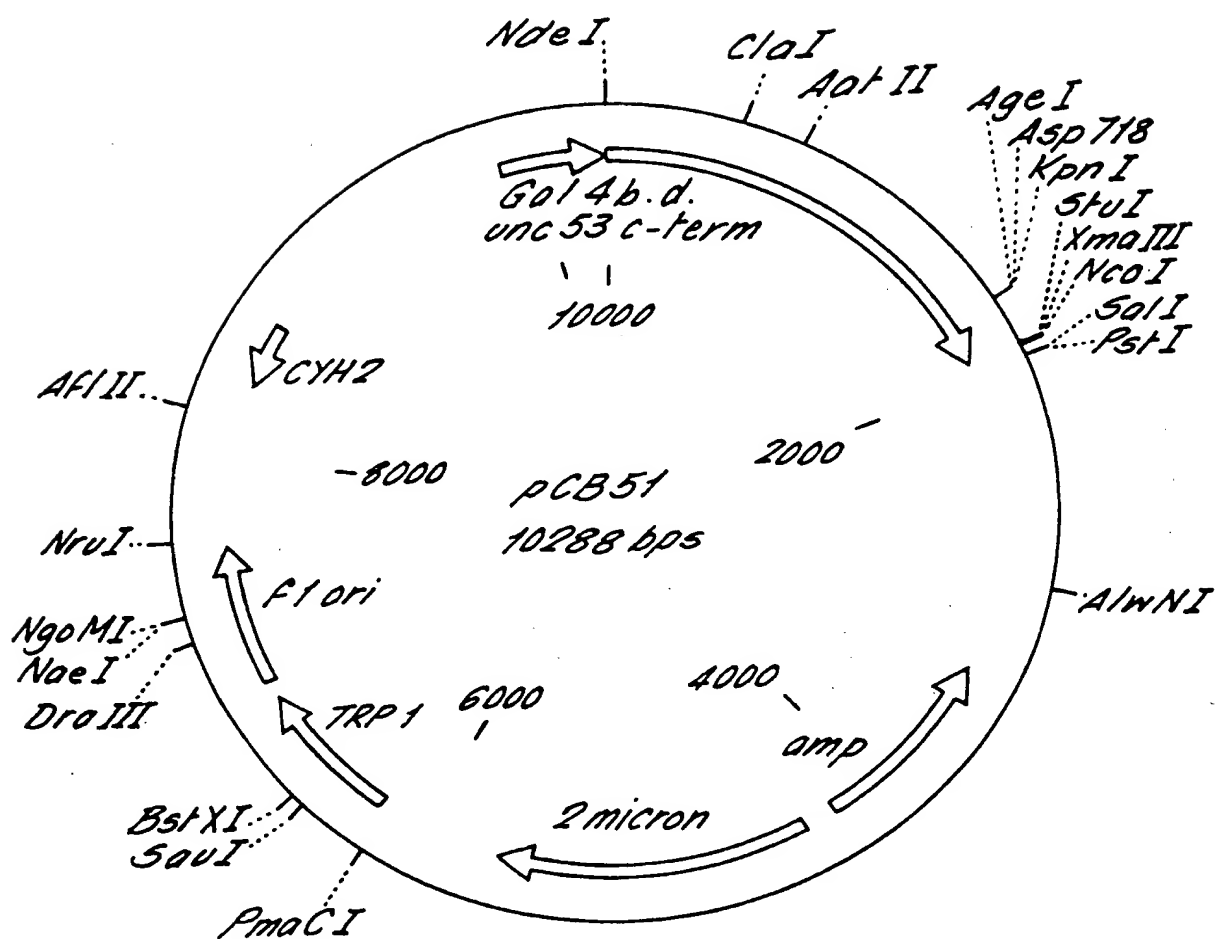
## FIG. 30 CONTINUED.

GTTTGCCGCT	TTGCTATCAA	GTATAAATAG	ACCTGCAATT	ATTAATCTTT	TGTTTCCTCG	12840
TCATTGTTCT	CGTTCCCTTT	CTTCCTTGTT	TCTTTTTCTG	CACAATATTT	CAAGCTATAC	12900
CAAGCATACA	ATCAACTCCA	AGCTTGAAGC	AAGCCTCCTG	AAAGATGAAG	CTACTGTCTT	12960
CTATCGAACA	AGCATGCGAT	ATTTGCCGAC	TTAAAAAGCT	CAAGTGCTCC	AAAGAAAAAC	13020
CGAAGTGCGC	CAAGTGTCTG	AAGAACAAC	GGGAGTGTCG	CTACTCTCCC	AAAACCAAAA	13080
GGTCTCCGCT	GACTAGGGCA	CATCTGACAG	AAGTGAATC	AAGGCTAGAA	AGACTGGAAC	13140
AGCTATTTCT	ACTGATTTTT	CCTCGAGAAG	ACCTTGACAT	GATTTTGAAA	ATGGATTCTT	13200
TACAGGATAT	AAAAGCATTG	TTAACAGGAT	TATTTGTACA	AGATAATGTG	AATAAAGATG	13260
CCGTCACAGA	TAGATTGGCT	TCAGTGGAGA	CTGATATGCC	TCTAACATTG	AGACAGCATA	13320
GAATAAGTGC	GACATCATCA	TCGGAAGAGA	GTAAGTAACAA	AGGTCAAAGA	CAGTTGACTG	13380
TATCGCCGGA	ATTGCAATAC	CCAGCTTTGA	CTCA			13414



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FIG. 31.



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FIG. 32.

TATGCCATCA	ATTTCCGGAT	CTCAAGGAAC	TCTTGACAAC	ATTGATGTGA	TTGAGTTGAA	60
GCAAGAGCTC	AAAGAACGCG	ATAGTGCACT	TTACGAAGTC	CGCCTTGACA	ATCTGGATCG	120
TGCCCCGCGAA	GTTGATGTTT	TGAGGGAGAC	AGTGAACAAG	TTGAAAACCG	AGAACAAGCA	180
ATTAAAGAAA	GAAGTGGACA	AACTCACCAA	CGGTCCAGCC	ACTCGTGCTT	CTTCCCGCGC	240
CTCAATTCCA	GTTATCTACG	ACGATGAGCA	TGTCTATGAT	GCAGCGTGTA	GCAGTACATC	300
AGCTAGTCAA	TCTTCGAAAC	GATCCTCTGG	CTGCAACTCA	ATCAAGGTTA	CTGTAAACGT	360
GGACATCGCT	GGAGAAATCA	GTTTCGATCGT	TAACCCGGAC	AAAGAGATAA	TCGTAGGATA	420
TCTTGCCATG	TCAACCAGTC	AGTCATGCTG	GAAAGACATT	GATGTTTCTA	TTCTAGGACT	480
ATTTGAAGTC	TACCTATCCA	GAATTGATGT	GGAGCATCAA	CTTGGAATCG	ATGCTCGTGA	540

*FIG. 32 CONTINUED.**75/99*

TTCTATCCTT GGCTATCAAA TTGGTGAAGT TCGACGCGTC ATTGGAGACT CCACAACCAT	600
GATAACCAGC CATCCAAGT ACATTCTTAC TTCCTCAACT ACAATCCGAA TGTTCATGCA	660
CGGTGCCGCA CAGAGTCGCG TAGACAGTCT GGTCTTGAT ATGCTTCTTC CAAAGCAAAT	720
GATTCTCCAA CTCGTCAAGT CAATTTTGAC AGAGAGACGT CTGGTGTTAG CTGGAGCAAC	780
TGGAATTGGA AAGAGCAAAC TGGCGAAGAC CCTGGCTGCT TATGTATCTA TTCGAACAAA	840
TCAATCCGAA GATAGTATTG TTAATATCAG CATTCTGAA AACAATAAAG AAGAATTGCT	900
TCAAGTGGA CGACGCCTGG AAAAGATCTT GAGAAGCAAA GAATCATGCA TCGTAATTCT	960
AGATAATATC CCAAAGAATC GAATTGCATT TGTTGTATCC GTTTTGCAA ATGTCCCACT	1020
TCAAAACAAC GAAGGTCCAT TTGTAGTATG CACAGTCAAC CGATATCAAA TCCCTGAGCT	1080
TCAAATTCAC CACAATTTCA AAATGTCAGT AATGTCGAAT CGTCTCGAAG GATTCATCCT	1140
ACGTTACCTC CGACGACGGG CGGTAGAGGA TGAGTATCGT CTAAGTGTAC AGATGCCATC	1200
AGAGCTCTTC AAAATCATTG ACTTCTTCCC AATAGCTCTT CAGGCCGTCA ATAATTTTAT	1260
TGAGAAAACG AATTCTGTTG ATGTGACAGT TGGTCCAAGA GCATGCTTGA ACTGTCCTCT	1320
AACTGTCGAT GGATCCCGTG AATGGTTCAT TCGATTGTGG AATGAGAACT TCATTCCATA	1380
TTTGGAACGT GTTGCTAGAG ATGGCAAAAA AACCTTCGGT CGCTGCACTT CCTTCGAGGA	1440
TCCCACCGAC ATCGTCTCTA AAAAATGGCC GTGGTTCGAT GGTGAAAACC CGGAGAATGT	1500
GCTCAAACGT CTTCAACTCC AAGACCTCGT CCCGTACCT GCCAACTCAT CCCGACAACA	1560
CTTCAATCCC CTCGAGTCGT TGATCCAATT GCATGCTACC AAGCATCAGA CCATCGACAA	1620
CATTTGAACA GAAGACTCTA ATCTTCTCTC GCCTCTCCCC CGCTTTCCTT ATCTTCGTAC	1680
CGGTACCTGA TGATTCCCCA TTTTCCCCCT TTTCCCCCA ATTTCCAGA ACCTCCTGTT	1740
CCCTTTGTTT CTAGTCCTCC CGGGTGCCGA CGCCGAAGCG ATTTAAAAAC CTTTTTCTTT	1800
CCGAAACATT TCCCATTGCT CATTAATAGT CAAATTGAAT AAACAGTGTA TGACTTAAA	1860
AAAAAAAAAA AAAAAAAAAA AAAAGGCCTA TGCGGCCGGG CCATGGAGGC CGAATTCCCG	1920
GGGATCCGTC GACCTGCAGC CAAGCTAATT CCGGGCGAAT TTCTTATGAT TTATGATTTT	1980
TATTATTAAA TAAGTTATAA AAAAAATAAG TGTATACAAA TTTTAAAGTG ACTCTTAGGT	2040
TTTAAACGA AAATTCTTGT TCTTGAGTAA CTCTTCTCG TAGGTCAGGT TGCTTTCTCA	2100
GGTATAGCAT GAGGTCGCTC TTATTGACCA CACCTCTACC GGCATGCAAG CTTGGCGTAA	2160
TCATGGTCAT AGCTGTTTCC TGTGTGAAAT TGTTATCCGC TCACAATTCC ACACAACATA	2220
CGAGCCGGAA GCATAAAGTG TAAAGCCTGG GGTGCCTAAT GAGTGAGGTA ACTCACATTA	2280
ATTGCGTTGC GCTCACTGCC CGCTTTCAG TCGGGAAACC TGTCGTGCCA GCTGGATTAA	2340
TGAATCGGCC AACGCGCGGG GAGAGGCGGT TTGCGTATTG GCGCTCTTC CGCTTCCTCG	2400
CTCACTGACT CGCTGCGCTC GGTCGTTCCG CTGCGGCGAG CGGTATCAGC TCACTCAAAG	2460

FIG. 32 CONTINUED.

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GCGGTAATAC	GGTTATCCAC	AGAATCAGGG	GATAACGCAG	GAAAGAACAT	GTGAGCAAAA	2520
GGCCAGCAAA	AGGCCAGGAA	CCGTAAAAAG	GCCGCGTTGC	TGGCGTTTTT	CCATAGGCTC	2580
CGCCCCCTG	ACGAGCATCA	CAAAAAATCGA	CGCTCAAGTC	AGAGGTGGCG	AAACCCGACA	2640
GGACTATAAA	GATACCAGGC	GTTTCCCCCT	GGAAGCTCCC	TCGTGCGCTC	TCCTGTTCCG	2700
ACCCTGCCGC	TTACCGGATA	CCTGTCCGCC	TTTCTCCCTT	CGGGAAGCGT	GGCGCTTTCT	2760
CATAGCTCAC	GCTGTAGGTA	TCTCAGTTCG	GTGTAGGTCG	TTGCTCCAA	GCTGGGCTGT	2820
GTGCACGAAC	CCCCCGTTCA	GCCCGACCGC	TGCGCCTTAT	CCGGTAACTA	TCGTCTTGAG	2880
TCCAACCCGG	TAAGACACGA	CTTATCGCCA	CTGGCAGCAG	CCACTGGTAA	CAGGATTAGC	2940
AGAGCGAGGT	ATGTAGGCGG	TGCTACAGAG	TTCTTGAAGT	GGTGGCCTAA	CTACGGCTAC	3000
ACTAGAAGGA	CAGTATTTGG	TATCTGCGCT	CTGCTGAAGC	CAGTTACCTT	CGGAAAAAGA	3060
GTTGGTAGCT	CTTGATCCGG	CAACAAACC	ACCCTGGTA	GCGGTGGTTT	TTTTGTTTGC	3120
AAGCAGCAGA	TTACGCGCAG	AAAAAAAGGA	TCTCAAGAAG	ATCCTTTGAT	CTTTTCTACG	3180
GGGTCTGACG	CTCAGTGGAA	CGAAAACTCA	CGTTAAGGGA	TTTTGGTCAT	GAGATTATCA	3240
AAAAGGATCT	TCACCTAGAT	CCTTTTAAAT	TAAAAATGAA	GTTTTAAATC	AATCTAAAGT	3300
ATATATGAGT	AAACTTGGTC	TGACAGTTAC	CAATGCTTAA	TCAGTGAGGC	ACCTATCTCA	3360
GCGATCTGTC	TATTTCGTTC	ATCCATAGTT	GCCTGACTCC	CCGTCGTGTA	GATAACTACG	3420
ATACGGGAGG	GCTTACCATC	TGGCCCCAGT	GCTGCAATGA	TACCGCGAGA	CCCACGCTCA	3480
CCGGCTCCAG	ATTTATCAGC	AATAAACCAG	CCAGCCGGAA	GGGCCGAGCG	CAGAAGTGGT	3540
CCTGCAACTT	TATCCGCCTC	CATCCAGTCT	ATTAATTGTT	GCCGGGAAGC	TAGAGTAAGT	3600
AGTTCGCCAG	TTAATAGTTT	GCGCAACGTT	GTTGCCATTG	CTACAGGCAT	CGTGGTGTCA	3660
CGCTCGTCGT	TTGGTATGGC	TTCAATCAGC	TCCGGTTCCC	AACGATCAAG	GCGAGTTACA	3720
TGATCCCCCA	TGTTGTGCAA	AAAAGCGGTT	AGCTCCTTCG	GTCCTCCGAT	CGTTGTGAGA	3780
AGTAAGTTGG	CCGCAGTGTT	ATCACTCATG	GTTATGGCAG	CACTGCATAA	TTCTCTTACT	3840
GTCATGCCAT	CCGTAAGATG	CTTTTCTGTG	ACTGGTGAGT	ACTCAACCAA	GTCATTCTGA	3900
GAATAGTGTA	TGCGGCGACC	GAGTTGCTCT	TGCCCCGGCGT	CAATACGGGA	TAATACCGCG	3960
CCACATAGCA	GAACTTTAAA	AGTGCTCATC	ATTGGAAAAC	GTTCTTCGGG	GCGAAAACCTC	4020
TCAAGGATCT	TACCGCTGTT	GAGATCCAGT	TCGATGTAAC	CCACTCGTGC	ACCCAACTGA	4080
TCTTCAGCAT	CTTTTACTTT	CACCAGCGTT	TCTGGGTGAG	CAAAAACAGG	AAGGCAAAAT	4140
GCCGCAAAAA	AGGGAATAAG	GGCGACACGG	AAATGTTGAA	TACTCATACT	CTTCCTTTTT	4200
CAATATTATT	GAAGCATTTA	TCAGGGTTAT	TGTCTCATGA	GCGGATACAT	ATTTGAATGT	4260
ATTTAGAAAA	ATAAACAAAT	AGGGGTTCCG	CGCACATTTT	CCCGAAAAGT	GCCACCTGAA	4320
CGAAGCATCT	GTGCTTCATT	TTGTAGAACA	AAAATGCAAC	GCGAGAGCGC	TAATTTTTCA	4380

*FIG. 32 CONTINUED.**77/99*

AACAAAGAAT CTGAGCTGCA TTTTACAGA ACAGAAATGC AACGCGAAAG CGCTATTTTA	4440
CCAACGAAGA ATCTGTGCTT CATTTTTGTA AAACAAAAT GCAACGCGAG AGCGCTAATT	4500
TTTCAAACAA AGAATCTGAG CTGCATTTTT ACAGAACAGA AATGCAACGC GAGAGCGCTA	4560
TTTTACCAAC AAAGAATCTA TACTTCTTTT TTGTTCTACA AAAATGCATC CCGAGAGCGC	4620
TATTTTTCTA ACAAAGCATC TTAGATTACT TTTTTCTCC TTTGTGCGCT CTATAATGCA	4680
GTCTCTTGAT AACTTTTTGC ACTGTAGGTC CGTTAAGGTT AGAAGAAGGC TACTTTGGTG	4740
TCTATTTTCT CTTCCATAAA AAAAGCCTGA CTCCACTTCC CGCGTTTACT GATTACTAGC	4800
GAAGCTGCGG GTGCATTTTT TCAAGATAAA GGCATCCCCG ATTATATTCT ATACCGATGT	4860
GGATTGCGCA TACTTTGTGA ACAGAAAGTG ATAGCGTTGA TGATTCTTCA TTGGTCAGAA	4920
AATTATGAAC GGTTCCTTCT ATTTTGTCTC TATATACTAC GTATAGGAAA TGTTTACATT	4980
TTCGTATTGT TTTCGATTCA CTCTATGAAT AGTTCTTACT ACAATTTTTT TGTCTAAAGA	5040
GTAATACTAG AGATAAACAT AAAAATGTA GAGGTCGAGT TTAGATGCAA GTTCAAGGAG	5100
CGAAAGGTGG ATGGGTAGGT TATATAGGGA TATAGCACAG AGATATATAG CAAAGAGATA	5160
CTTTTGAGCA ATGTTTGTGG AAGCGGTATT CGCAATATTT TAGTAGCTCG TTACAGTCCG	5220
GTGCGTTTTT GGTTTTTTGA AAGTGCGTCT TCAGAGCGCT TTTGGTTTTT AAAAGCGCTC	5280
TGAAGTTCCT ATACTTTCTA GAGAATAGGA ACTTCGGAAT AGGAACTTCA AAGCGTTTTCC	5340
GAAAACGAGC GCTTCCGAAA ATGCAACGCG AGCTGCGCAC ATACAGCTCA CTGTTACAGT	5400
CGCACCTATA TCTGCGTGT GCCTGTATAT ATATATACAT GAGAAGAACG GCATAGTGCG	5460
TGTTTATGCT TAAATGCGTA CTTATATGCG TCTATTTATG TAGGATGAAA GGTAGTCTAG	5520
TACCTCCTGT GATATTATCC CATTCCATGC GGGGTATCGT ATGCTTCCTT CAGCACTACC	5580
CTTTAGCTGT TCTATATGCT GCCACTCCTC AATTGGATTA GTCTCATCCT TCAATGCTAT	5640
CATTTCTTTT GATATTGGAT CATATTAAGA AACCATTATT ATCATGACAT TAACCTATAA	5700
AAATAGGCGT ATCACGAGGC CCTTTCGTCT CGCGCGTTTC GGTGATGACG GTGAAAACCT	5760
CTGACACATG CAGCTCCCGG AGACGGTCAC AGCTTGTCTG TAAGCGGATG CCGGGAGCAG	5820
ACAAGCCCGT CAGGGCGCGT CAGCGGGTGT TGGCGGGTGT CGGGGCTGGC TTAACATATGC	5880
GGCATCAGAG CAGATTGTAC TGAGAGTGCA CCATAGATCA ACGACATTAC TATATATATA	5940
ATATAGGAAG CATTTAATAG ACAGCATCGT AATATATGTG TACTTTGCAG TTATGACGCC	6000
AGATGGCAGT AGTGGAAGAT ATTCTTTATT GAAAAATAGC TTGTCACCTT ACGTACAATC	6060
TTGATCCGGA GCTTTTCTTT TTTTGCCGAT TAAGAATTAA TTCGGTCGAA AAAAGAAAAG	6120
GAGAGGGCCA AGAGGGAGGG CATTGGTGAC TATTGAGCAC GTGAGTATAC GTGATTAAGC	6180
ACACAAAGGC AGCTTGAGT ATGTCTGTTA TTAATTTTAC AGGTAGTTCT GGTCCATTGG	6240
TGAAAGTTTG CGGCTTGAG AGCACAGAGG CCGCAGAATG TGCTCTAGAT TCCGATGCTG	6300

FIG. 32 CONTINUED.

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ACTTGCTGGG TATTATATGT GTGCCCAATA GAAAGAGAAC AATTGACCCG GTTATTGCAA	6360
GGAAAATTTTC AAGTCTTGTA AAAGCATATA AAAATAGTTC AGGCACTCCG AAATACTTGG	6420
TTGGCGTGTT TCGTAATCAA CCTAAGGAGG ATGTTTTTGGC TCTGGTCAAT GATTACGGCA	6480
TTGATATCGT CCAACTGCGT GGAGATGAGT CGTGGAAGA ATACCAAGAG TTCCTCGGTT	6540
TGCCAGTTAT TAAAGACTC GTATTTCCAA AAGACTGCAA CATACTACTC AGTGCAGCTT	6600
CACAGAAACC TCATTCGTTT ATTCCCTTGT TTGATTCAGA AGCAGGTGGG ACAGGTGAAC	6660
TTTTGGATTG GAACTCGATT TCTGACTGGG TTGGAAGGCA AGAGAGCCCC GAAAGCTTAC	6720
ATTTTATGTT AGCTGGTGGG CTGACGCCAG AAAATGTTGG TGATGCGCTT AGATTAAATG	6780
GCGTTATTGG TGTTGATGTA AGCGGAGGTG TGGAGACAAA TGGTGTAAAA GACTCTAACA	6840
AAATAGCAAA TTTCGTCAA AATGCTAAGA AATAGGTTAT TACTGAGTAG TATTTATTTA	6900
AGTATTGTTT GTGCACTTGC CGATCTATGC GGTGTGAAAT ACCGCACAGA TCGTAAGGA	6960
GAAAATACCG CATCAGGAAA TTGTAAACGT TAATATTTTG TTAAAATTCG CGTTAAATTT	7020
TTGTTAAATC AGCTCATTTT TTAACCAATA GGCCGAAATC GGCAAAATCC CTTATAAATC	7080
AAAAGAATAG ACCGAGATAG GGTGAGTGT TGTTCCAGTT TGGAACAAGA GTCCACTATT	7140
AAAGAACGTG GACTCCAACG TCAAAGGGCG AAAAACCCTC TATCAGGGCG ATGGCCCACT	7200
ACGTGAACCA TCACCCTAAT CAAGTTTTTT GGGGTCGAGG TGCCGTAAAG CACTAAATCG	7260
GAACCCTAAA GGGAGCCCC GATTTAGAGC TTGACGGGGA AAGCCGGCGA ACGTGGCGAG	7320
AAAGGAAGGG AAGAAAGCGA AAGGAGCGGG CGCTAGGGCG CTGGCAAGTG TAGCGGTCAC	7380
GCTGCGCGTA ACCACCACAC CCGCCGCGCT TAATGCGCCG CTACAGGGCG CGTCGCGCCA	7440
TTCGCCATTC AGGCTGCGCA ACTGTTGGGA AGGGCGATCG GTGCGGGCCT CTTGCTATT	7500
ACGCCAGCTG GCGAAAGGGG GATGTGCTGC AAGGCGATTA AGTTGGGTAA CGCCAGGGTT	7560
TTCCAGTCA CGACGTTGTA AAACGACGGC CAGTCGTCCA AGCTTTCGCG AGCTCGAGAT	7620
CCCAGCTTT GCAAATTAAA GCCTTCGAGC GTCCCAAAAC CTTCTCAAGC AAGGTTTTCA	7680
GTATAATGTT ACATGCGTAC ACGCGTCTGT ACAGAAAAAA AAGAAAAATT TGAAATATAA	7740
ATAACGTTCT TAATACTAAC ATAACATAA AAAAATAAAT AGGGACCTAG ACTTCAGGTT	7800
GTCTAACTCC TTCCTTTTCG GTTAGAGCGG ATGTGGGGGG AGGGCGTGAA TGTAAGCGTG	7860
ACATAACTAA TTACATGATA TCGACAAAGG AAAAGGGGCC TGTTTACTCA CAGGCTTTTT	7920
TCAAGTAGGT AATTAAGTCG TTTCTGTCTT TTTCCTTCTT CAACCCACCA AAGGCCATCT	7980
TGGTACTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT	8040
TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT CATAGAAATA ATACAGAAGT	8100
AGATGTTGAA TTAGATTAAA CTGAAGATAT ATAATTTATT GGAAAATACA TAGAGCTTTT	8160
TGTTGATGCG CTTAAGCGAT CAATTCACCA ACACCACCAG CAGCTCTGAT TTTTCTTCA	8220

FIG. 32 CONTINUED.

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GCCAACTTGG AGACGAATCT AGCTTTGACG ATAAGTGGAA CATTGTTGGAT TCTACCCTTA	8280
CCCAAGATCT TACCGTAACC GGCTGCCAAA GTGTCAATAA CTGGAGCAGT TTCCTTAGAA	8340
GCAGATTTCA AGTATTGGTC TCTCTTGTCT TCTGGGATCA ATGTCCACAA TTTGTCCAAG	8400
TTCAAGACTG GCTTCCAGAA ATGAGCTTGT TGCTTGTGGA AGTATCTCAT ACCAANCCTT	8460
ACCGAAATAA CCTGGATGGT ATTTATCCAT GTTAATTCTG TGGTGATGTT GACCACCGGC	8520
CATACCTCTA CCACCGGGGT GCTTTCTGTG CTTACCGATA CGACCTTTAC CGGCTGAGAC	8580
GTGACCTCTG TGCTTTCTAG TCTTAGTGAA TCTGGAAGGC ATTCTTGATT AGTTGGATGA	8640
TTGTTCTGGG ATTTAATGCA AAAAAATCAC TAAGAAGGAA AAAAAATCAAC GGAGAAAGCA	8700
AACGCCATCT TAAATATACG GGATACAGAT GAAAGGTTTG AACCTATCTG GGAAAATACG	8760
CATTAAACAA GCGAAAACT GCGAGGAAAA TTGTTTGCGT CTCTGCGGGC TATTCACGCG	8820
CCAGAGGAAA ATAGGAAAAA TAACAGGGCA TTAGAAAAAT AATTTTGATT TTGGTAATGT	8880
GTGGGTCCCT GGTGTACAGA TGTTACATTG GTTACAGTAC TCTTGTTTTT GCTGTGTTTT	8940
TCGATGAATC TCCAAAATGG TTGTTAGCAC ATGGAAGAGT CACCGATGCT AAGTTATCTC	9000
TATGTAAGCT ACGTGGCGTG ACTTTTGATG AAGCCGCACA AGAGATACAG GATTGGCAAC	9060
TGCAAATAGA ATCTGGGGAT CTAGATATCC TTTTGTTGTT TCCGGGTGTA CAATATGGAC	9120
TTCTCTTTTT CTGGCAACCA AACCCATACA TCGGGATTCC TATAATACCT TCGTTGGTCT	9180
CCCTAACATG TAGGTGGCGG AGGGGAGATA TACAATAGAA CAGATACCAG ACAAGACATA	9240
ATGGGCTAAA CAAGACTACA CCAATTACAC TGCTTCATTG ATGGTGGTAC ATAACGAAC	9300
AATACTGTAG CCCTAGACTT GATAGCCATC ATCATATCGA AGTTTCACTA CCCTTTTTCC	9360
ATTTGCCATC TATTGAAGTA ATAATAGGCG CATGCAACTT CTTTTCTTTT TTTTCTTTTT	9420
CTCTCTCCCC CGTTGTTGTC TCACCATATC CGCAATGACA AAAAAAATGA TGGAAGACAC	9480
TAAAGGAAAA AATTAACGAC AAAGACAGCA CCAACAGATG TCGTTGTTCC AGAGCTGATG	9540
AGGGGTATCT TCGAACACAC GAACTTTTTT CCTTCCTTCA TTCACGCACA CTACTCTCTA	9600
ATGAGCAACG GTATACGGCC TTCCTTCCAG TTAAGTGAAT TTGAAATAAA AAAAGTTTGC	9660
CGCTTTGCTA TCAAGTATAA ATAGACCTGC AATTATTAAT CTTTTGTTTC CTCGTCATTG	9720
TTCTCGTTCC CTTTCTTCCT TGTTTCTTTT TCTGCACAAT ATTTCAAGCT ATACCAAGCA	9780
TACAATCAAC TCCAAGCTTG AAGCAAGCCT CCTGAAAGAT GAAGCTACTG TCTTCTATCG	9840
AACAAGCATG CGATATTTGC CGACTTAAAA AGCTCAAGTG CTCCAAAGAA AAACCGAAGT	9900
GCGCCAAGTG TCTGAAGAAC AACTGGGAGT GTCGCTACTC TCCCAAACC AAAAGGTCTC	9960
CGCTGACTAG GGCACATCTG ACAGAAGTGG AATCAAGGCT AGAAAGACTG GAACAGCTAT	10020
TTCTACTGAT TTTTCTTCGA GAAGACCTTG ACATGATTTT GAAAATGGAT TCTTTACAGG	10080
ATATAAAGC ATTGTTAACA GGATTATTTG TACAAGATAA TGTGAATAAA GATGCCGTCA	10140

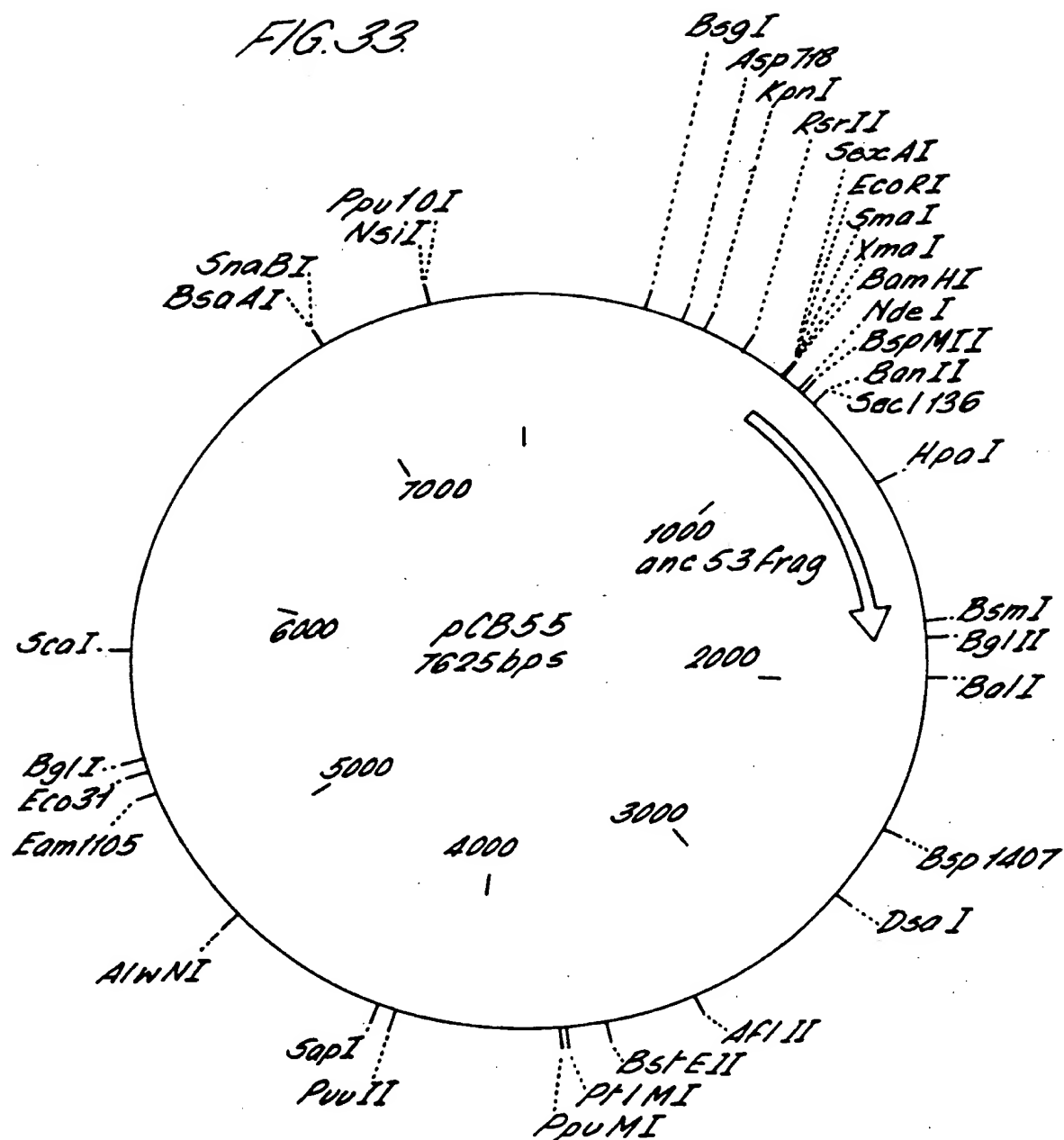
*80/99**FIG. 32 CONTINUED.*

CAGATAGATT GGCTTCAGTG GAGACTGATA TGCCTCTAAC ATTGAGACAG CATAGAATAA	10200
GTGCGACATC ATCATCGGAA GAGAGTAGTA ACAAAGGTCA AAGACAGTTG ACTGTATCGC	10260
CGGAATTGCA ATACCCAGCT TTGACTCA	10288



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FIG. 33



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FIG. 34.

GCTTGCAATGC AACTTCTTTT CTTTTTTTTT CTTTCTCTC TCCCCCGTTG TTGTCTCACC	60
ATATCCGCAA TGACAAAAA AATGATGGAA GACACTAAAG GAAAAAATTA ACGACAAAGA	120
CAGCACCAAC AGATGTCGTT GTTCCAGAGC TGATGAGGGG TATCTTCGAA CACACGAAAC	180
TTTTTCCTTC CTTCAATTCAC GCACACTACT CTCTAATGAG CAACGGTATA CGGCCTTCCT	240
TCCAGTTACT TGAATTTGAA ATAAAAAAG TTTGCCGCTT TGCTATCAAG TATAAATAGA	300
CCTGCAATTA TTAATCTTTT GTTTCCTCGT CATTGTTCTC GTTCCCTTTC TTCCTTGTTT	360
CTTTTTCTGC ACAATATTTT AAGCTATACC AAGCATACAA TCAACTCCAA GCTTTGCAAA	420
GATGGATAAA GCGGAATTAA TTCCCGAGCC TCCAAAAAG AAGAGAAAGG TCGAATTGGG	480
TACCGCCGCC AATTTTAATC AAAGTGGGAA TATTGCTGAT AGCTCATTGT CTTCACTTT	540
CACTAACAGT AGCAACGGTC CGAACCTCAT AACAACCTCA ACAAATTCTC AAGCGCTTTC	600
ACAACCAATT GCCTCCTCTA ACGTTCATGA TAACTTCATG AATAATGAAA TCACGGCTAG	660
TAAAATTGAT GATGGTAATA ATTCAAACC ACTGTCACCT GGTGGACGG ACCAACTGC	720
GTATAACGCG TTTGGAATCA CTACAGGGAT GTTTAATACC ACTACAATGG ATGATGTATA	780
TAACTATCTA TTCGATGATG AAGATACCCC ACCAAACCCA AAAAAAGAGA TCGAATTCCC	840
GGGGATCCGC TCCTCACTCT CCAAGTTCAC CAAGAAGAAG AACAAGAAGT ACGACGAAGC	900
ACATATGCCA TCAATTTCCG GATCTCAAGG AACTCTTGAC AACATTGATG TGATTGAGTT	960
GAAGCAAGAG CTCAAAGAAC GCGATAGTGC ACTTTACGAA GTCCGCCTTG ACAATCTGGA	1020
TCGTGCCCCG GAAGTTGATG TTCTGAGGGA GACAGTGAAC AAGTTGAAAA CCGAGAACAA	1080
GCAATTAAAG AAAGAAGTGG ACAAACTCAC CAACGGTCCA GCCACTCGTG CTTCTTCCCG	1140
CGCCTCAATT CCAGTTATCT ACGACGATGA GCATGTCTAT GATGCAGCGT GTAGCAGTAC	1200

*FIG. 34 CONTINUED.**83/99*

ATCAGCTAGT CAATCTTCGA AACGATCCTC TGGCTGCAAC TCAATCAAGG TTA CTGTAAA	1260
CGTGGACATC GCTGGAGAAA TCAGTTCGAT CGTTAACCCG GACAAAGAGA TAATCGTAGG	1320
ATATCTTGCC ATGTCAACCA GTCAGTCATG CTGGAAAGAC ATTGATGTTT CTATTCTAGG	1380
ACTATTTGAA GTCTACCTAT CCAGAATTGA TGTGGAGCAT CAACTTGGAA TCGATGCTCG	1440
TGATTCTATC CTTGGCTATC AAATTGGTGA ACTTCGACGC GTCATTGGAG ACTCCACAAC	1500
CATGATAACC AGCCATCCAA CTGACATTCT TACTTCCTCA ACTACAATCC GAATGTTTAT	1560
GCACGGTGCC GCACAGAGTC GCGTAGACAG TCTGGTCCTT GATATGCTTC TTCCAAAGCA	1620
AATGATTCTC CAACTCGTCA AGTCAATTTT GACAGAGAGA CGTCTGGTGT TAGCTGGAGC	1680
AACTGGAATT GGAAAGAGCA AACTGGCGAA GACCCTGGCT GCTTATGTAT CTATTCGAAC	1740
AAATCAATCC GAAGATAGTA TTGTTAATAT CAGCATTCTT GAAAACAATA AAGAAGAATT	1800
GCTTCAAGTG GAACGACGCC TGGAAAAGAT CTATGAATCG TAGATACTGA AAAACCCCGC	1860
AAGTTCACCT CAACTGTGCA TCGTGCACCA TCTCAATTTT TTTCAATTTT ACATCGTTTT	1920
GCCTTCTTTT ATGTAACCTAT ACTCCTCTAA GTTTCAATCT TGGCCATGTA ACCTCTGATC	1980
TATAGAATTT TTTAAATGAC TAGAATTAAT GCCCATCTTT TTTTGGACC TAAATCTTC	2040
ATGAAAATAT ATTACGAGGG CTTATTCAGA AGCTTTGGAC TTCTTCGCCA GAGGTTTGGT	2100
CAAGTCTCCA ATCAAGGTTG TCGGCTTGTC TACCTTGCCA GAAATTTACG AAAAGATGGA	2160
AAAGGGTCAA ATCGTTGGTA GATACGTTGT TGACACTTCT AAATAAGCGA ATTTCTTATG	2220
ATTTATGATT TTTATTATTA AATAAGTTAT AAAAAAATA AGTGATACA AATTTTAAAG	2280
TGACTCTTAG GTTTTAAAC GAAAATTCTT GTTCTTGAGT AACTCTTTCC TGTAGGTCAG	2340
GTGCTTTCT CAGGTATAGC ATGAGGTCGC TCTTATTGAC CACACCTCTA CCGGCATGCC	2400
CGAAATTCCC CTACCCTATG AACATATTCC ATTTTGTAAAT TTCGTGTCGT TTCTATTATG	2460
AATTTCAATTT ATAAAGTTTA TGTACAAATA TCATAAAAAA AGAGAATCTT TTTAAGCAAG	2520
GATTTTCTTA ACTTCTTCGG CGACAGCATC ACCGACTTCG GTGGTACTGT TGGAACCACC	2580
TAAATCACCA GTTCTGATAC CTGCATCCAA AACCTTTTTA ACTGCATCTT CAATGGCCTT	2640
ACCTTCTTCA GGCAAGTTCA ATGACAATTT CAACATCATT GCAGCAGACA AGATAGTGGC	2700
GATAGGGTCA ACCTTATTCT TTGGCAAATC TGGAGCAGAA CCGTGGCATG GTTCGTACAA	2760
ACCAAATGCG GTGTTCTTGT CTGGCAAAGA GGCCAAGGAC GCAGATGGCA ACAAACCCAA	2820
GGAACCTGGG ATAACGGAGG CTTCATCGGA GATGATATCA CCAACATGT TGCTGGTGAT	2880
TATAATACCA TTTAGGTGGG TTGGGTTCTT AACTAGGATC ATGGCGGCAG AATCAATCAA	2940
TTGATGTTGA ACCTTCAATG TAGGAAATTC GTTCTTGATG GTTTCCTCCA CAGTTTTTCT	3000
CCATAATCTT GAAGAGGCCA AAACATTAGC TTTATCCAAG GACCAAATAG GCAATGGTGG	3060
CTCATGTTGT AGGGCCATGA AAGCGGCCAT TCTTGTGATT CTTTGCACTT CTGGAACGGT	3120

*FIG. 34 CONTINUED.**84/99*

GTATTGTTCA CTATCCCAAG CGACACCATC ACCATCGTCT TCCTTTCTCT TACCAAAGTA	3180
AATACCTCCC ACTAATTCTC TGACAACAAC GAAGTCAGTA CCTTTAGCAA ATTGTGGCTT	3240
GATTGGAGAT AAGTCTAAAA GAGAGTCGGA TGCAAAGTTA CATGGTCTTA AGTTGGCGTA	3300
CAATTGAAGT TCTTTACGGA TTTTATAGTAA ACCTTGTTCA GGTCTAACAC TACCTGTACC	3360
CCATTTAGGA CCACCCACAG CACCTAACAA AACGGCATCA ACCTTCTTGG AGGCTTCCAG	3420
CGCCTCATCT GGAAGTGGGA CACCTGTAGC ATCGATAGCA GCACCACCAA TTAAATGATT	3480
TTCGAAATCG AACTTGACAT TGGAACGAAC ATCAGAAATA GCTTTAAGAA CCTTAATGGC	3540
TTCGGCTGTG ATTTCTTGAC CAACGTGGTC ACCTGGCAA ACGACGATCT TCTTAGGGGC	3600
AGACATTAGA ATGGTATATC CTTGAAATAT ATATATATAT TGCTGAAATG TAAAAGGTAA	3660
GAAAAGTTAG AAAGTAAGAC GATTGCTAAC CACCTATTGG AAAAAACAAT AGGTCCTTAA	3720
ATAATATTGT CAACTTCAAG TATTGTGATG CAAGCATTTA GTCATGAACG CTTCTCTATT	3780
CTATATGAAA AGCCGGTTCC GGCCTCTCAC CTTTCCTTTT TCTCCCAATT TTTCAATTGA	3840
AAAAGGTATA TGCCTCAGGC GACCTCTGAA ATTAACAAAA AATTTCCAGT CATCGAATTT	3900
GATTCTGTGC GATAGCGCCC CTGTGTGTTT TCGTTATGTT GAGGAAAAAA ATAATGGTTG	3960
CTAAGAGATT CGAACTCTTG CATCTTACGA TACCTGAGTA TTCCACAGT TGGGGATCTC	4020
GACTCTAGCT AGAGGATCAA TTCGTAATCA TGGTCATAGC TGTTTCCTGT GTGAAATTGT	4080
TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA TAAAGTGTA AGCCTGGGGT	4140
GCCTAATGAG TGAGGTA ACT CACATTAATT GCGTTGCGCT CACTGCCCCG TTTCCAGTCG	4200
GGAAACCTGT CGTGCCAGCT GGATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG	4260
CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TCGCTCGGT CGTTCGGCTG	4320
CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT	4380
AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC	4440
GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC	4500
TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT TCCCCCTGGA	4560
AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT	4620
CTCCCTTCGG GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT CAGTTCGGTG	4680
TAGGTCGTTT GCTCCAAGCT GGGCTGTGTG CACGAACCCC CCGTTCAGCC CGACCGCTGC	4740
GCCTTATCCG GTAACATATCG TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG	4800
GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC	4860
TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG TATTTGGTAT CTGCGCTCTG	4920
CTGAAGCCAG TTACCTTCGG AAAAAAGAGTT GGTAGCTCTT GATCCGGCAA ACAAACCACC	4980
GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA CGCGCAGAAA AAAAGGATCT	5040

*FIG. 34 CONTINUED.**85/99*

CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACCTCACGT	5100
TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTAAATTAA	5160
AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA CTTGGTCTGA CAGTTACCAA	5220
TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTTCATC CATAGTTGCC	5280
TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT	5340
GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA	5400
GCCGGAAGGG CCGAGCGCAG AAGTGGTCTT GCAACTTTAT CCGCCTCCAT CCAGTCTATT	5460
AATTGTTGCC GGGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG CAACGTTGTT	5520
GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTACAGCTCC	5580
GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCATGT TGTGCAAAAA AGCGGTTAGC	5640
TCCTTCGGTC CTCCGATCGT TGTCAAGAGT AAGTTGGCCG CAGTGTTATC ACTCATGGTT	5700
ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT TTCTGTGACT	5760
GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GCGACCGAG TTGCTCTTGC	5820
CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT	5880
GGAAAACGTT CTTCGGGGCG AAAACTCTCA AGGATCTTAC CGCTGTTGAG ATCCAGTTCG	5940
ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTTCA CAGCGTTTCT	6000
GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA	6060
TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA GCATTATCA GGGTTATTGT	6120
CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCGCGC	6180
ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA TTATTATCAT GACATTAACC	6240
TATAAAAATA GCGGTATCAC GAGGCCCTTT CGTCTCGCGC GTTTCGGTGA TGACGGTGAA	6300
AACCTCTGAC ACATGCAGCT CCCGGAGACG GTCACAGCTT GTCTGTAAGC GGATGCCGGG	6360
AGCAGACAAG CCCGTCAGGG CGCGTCAGCG GGTGTTGGCG GGTGTCGGGG CTGGCTTAAC	6420
TATGCGGCAT CAGAGCAGAT TGTACTGAGA GTGCACCATA ACGCATTAA GCATAAACAC	6480
GCACTATGCC GTTCTTCTCA TGTATATATA TATACAGGCA ACACGCAGAT ATAGGTGCGA	6540
CGTGAACAGT GAGCTGTATG TGCGCAGCTC GCGTTGCATT TTCGGAAGCG CTCGTTTTTCG	6600
GAAACGCTTT GAAGTTCCTA TTCCGAAGTT CCTATTCTCT AGCTAGAAAG TATAGGAACT	6660
TCAGAGCGCT TTTGAAAACC AAAAGCGCTC TGAAGACGCA CTTTCAAAAA ACCAAAAACG	6720
CACCGGACTG TAACGAGCTA CTAAATATT GCGAATACCG CTTCCACAAA CATTGCTCAA	6780
AAGTATCTCT TTGCTATATA TCTCTGTGCT ATATCCCTAT ATAACCTACC CATCCACCTT	6840
TCGCTCCTTG AACTTGCATC TAAACTCGAC CTCTACATTT TTTATGTTTA TCTCTAGTAT	6900
TACTCTTTAG ACAAAAAAAT TGTAGTAAGA ACTATTCTATA GAGTGAATCG AAAACAATAC	6960

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## FIG. 34 CONTINUED.

GAAAATGTAA ACATTTCTTA TACGTAGTAT ATAGAGACAA AATAGAAGAA ACCGTTTATA	7020
ATTTTCTGAC CAATGAAGAA TCATCAACGC TATCACTTTC TGTCACAAA GTATGCGCAA	7080
TCCACATCGG TATAGAATAT AATCGGGGAT GCCTTTATCT TGAAAAAATG CACCCGCAGC	7140
TTCGCTAGTA ATCAGTAAAC GCGGGAAGTG GAGTCAGGCT TTTTTTATGG AAGAGAAAAT	7200
AGACACCAAA GTAGCCTTCT TCTAACCTTA ACGGACCTAC AGTGCAAAAA GTTATCAAGA	7260
GACTGCATTA TAGAGCGCAC AAAGGAGAAA AAAAGTAATC TAAGATGCTT TGTTAGAAAA	7320
ATAGCGCTCT CGGGATGCAT TTTTGTAGAA CAAAAAGAA GTATAGATTC TTTGTTGGTA	7380
AAATAGCGCT CTCGCGTTGC ATTTCTGTTT TGTA AAAATG CAGCTCAGAT TCTTTGTTTG	7440
AAAAATTAGC GCTCTCGCGT TGCATTTTTG TTTTACAAA ATGAAGCACA GATTCTTCGT	7500
TGGTAAAATA GCGCTTTTCG GTTGCATTTT TGTTCTGTAA AAATGCAGCT CAGATTCTTT	7560
GTTTGAAAAA TTAGCGCTCT CGCGTTGCAT TTTTGTTCTA CAAAATGAAG CACAGATGCT	7620
TCGTT	7625

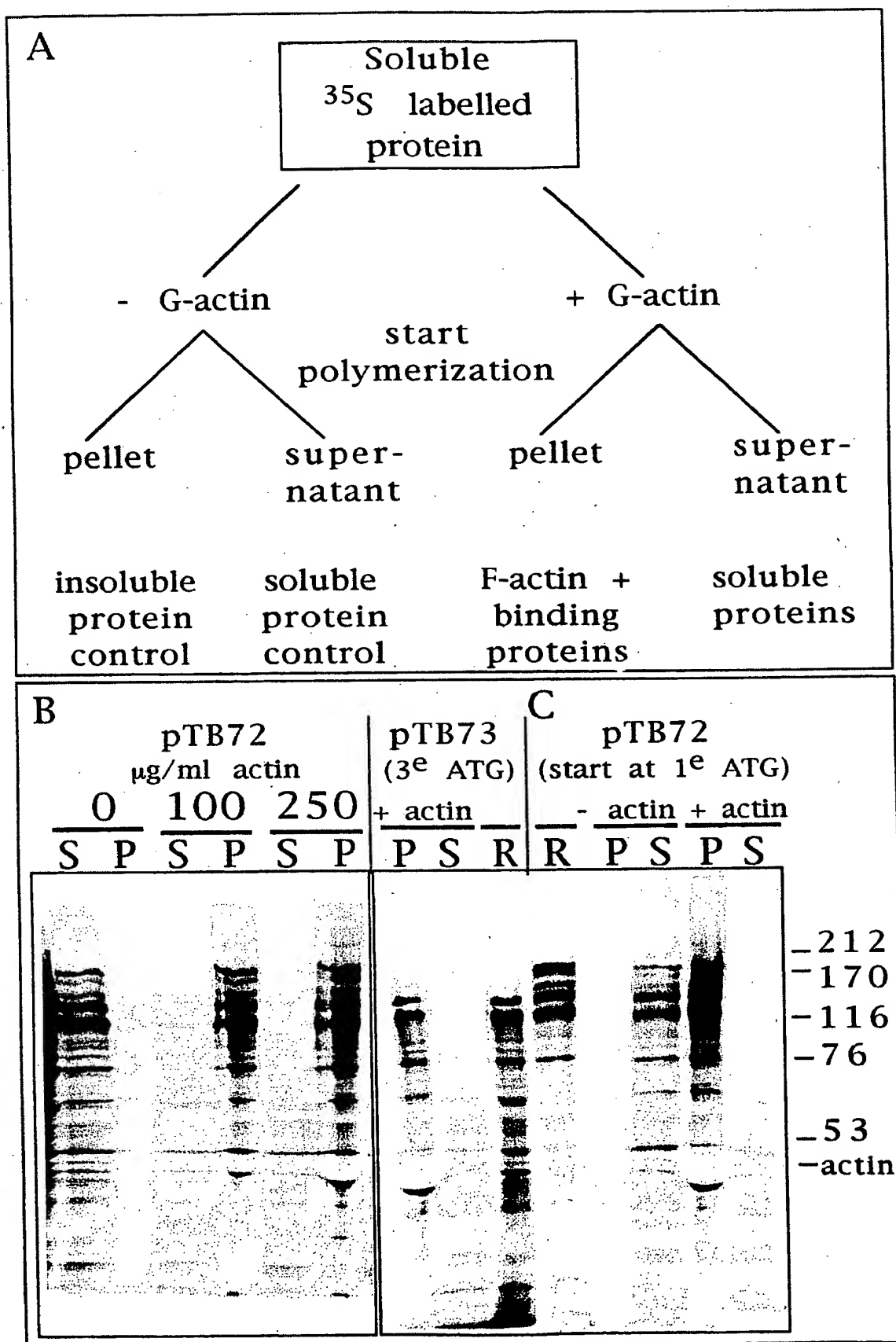
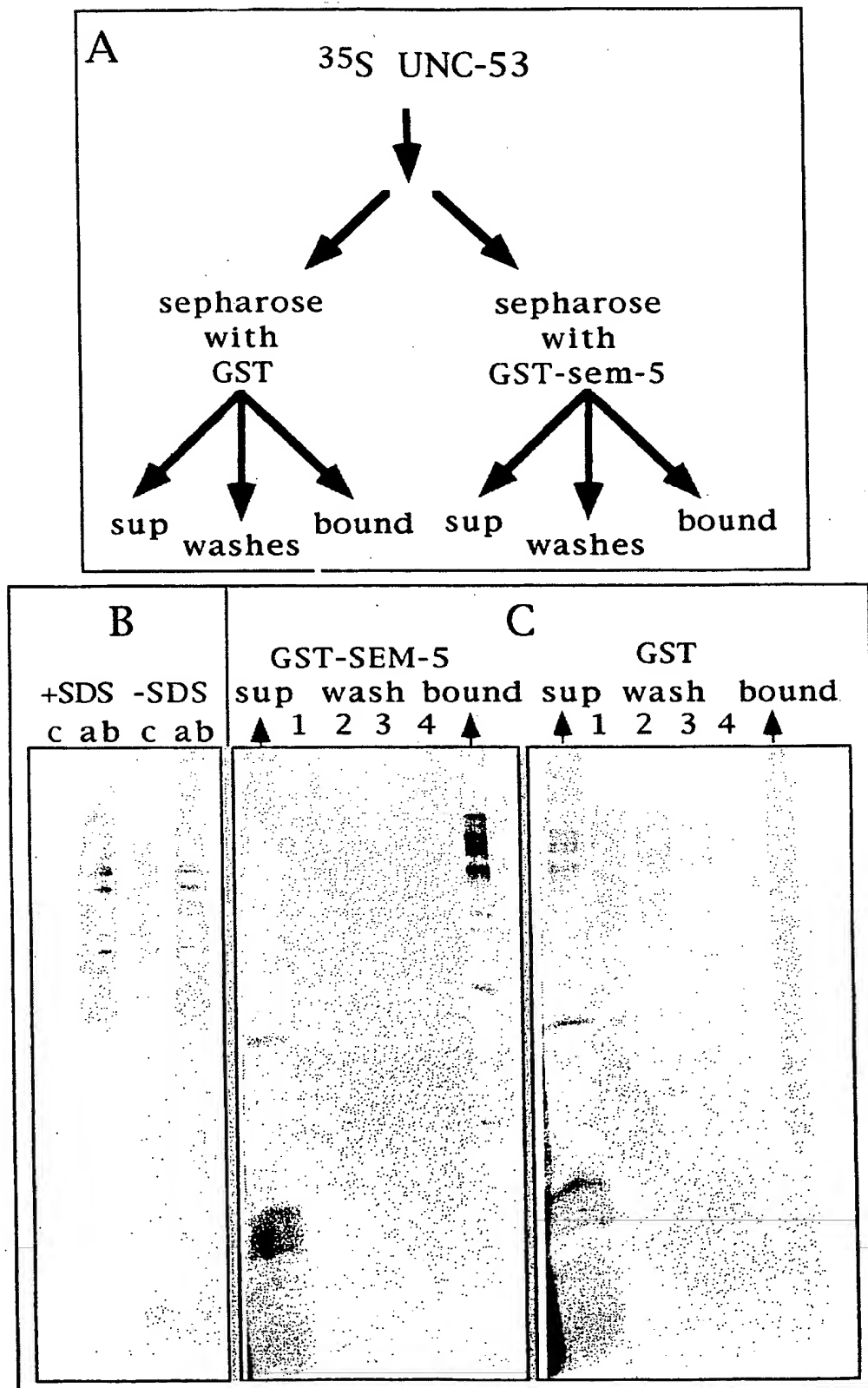


FIG. 35.

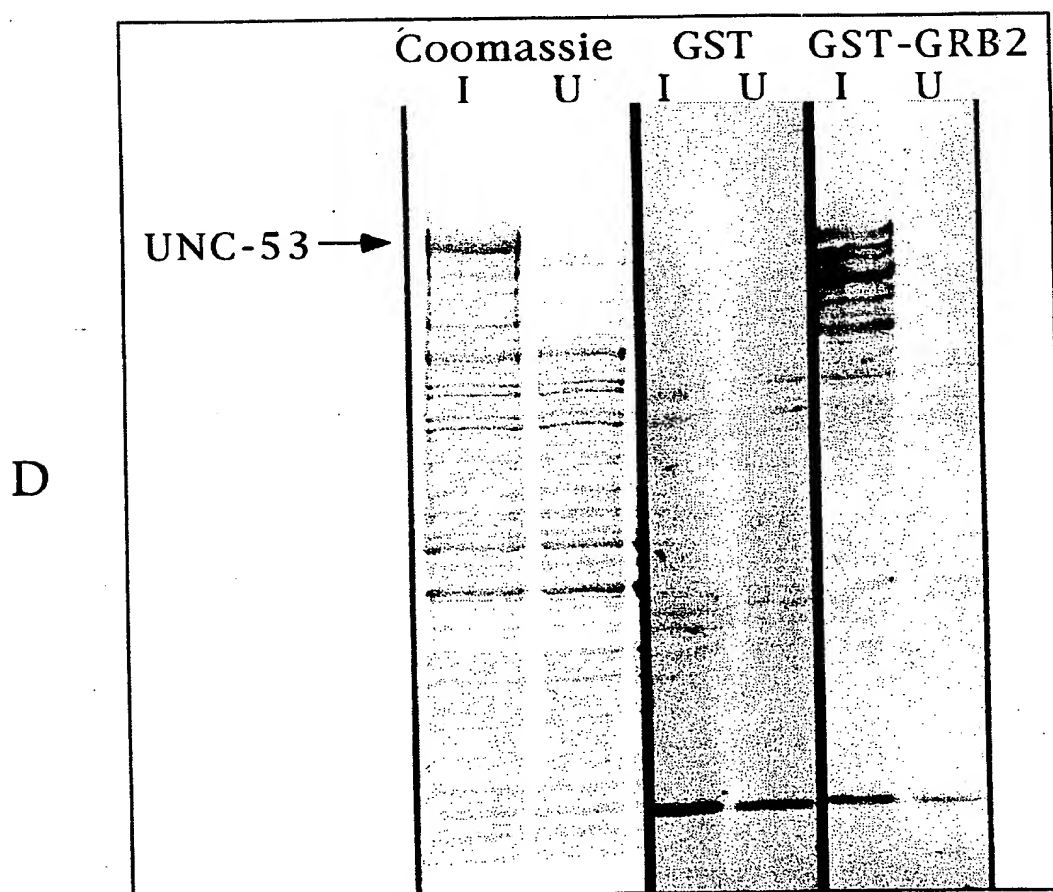
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FIG. 36.



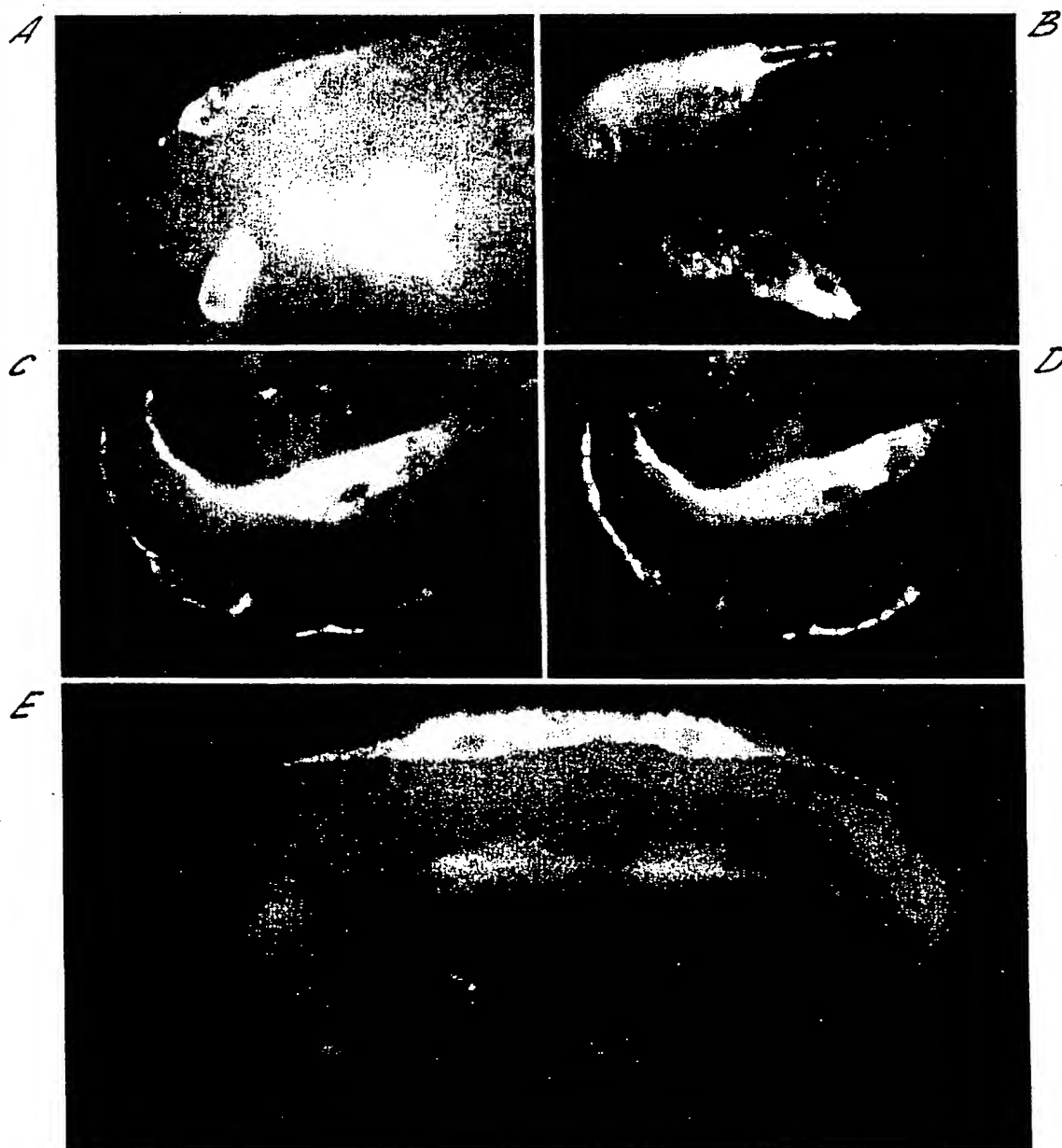


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*FIG. 36 (CONTD.)*

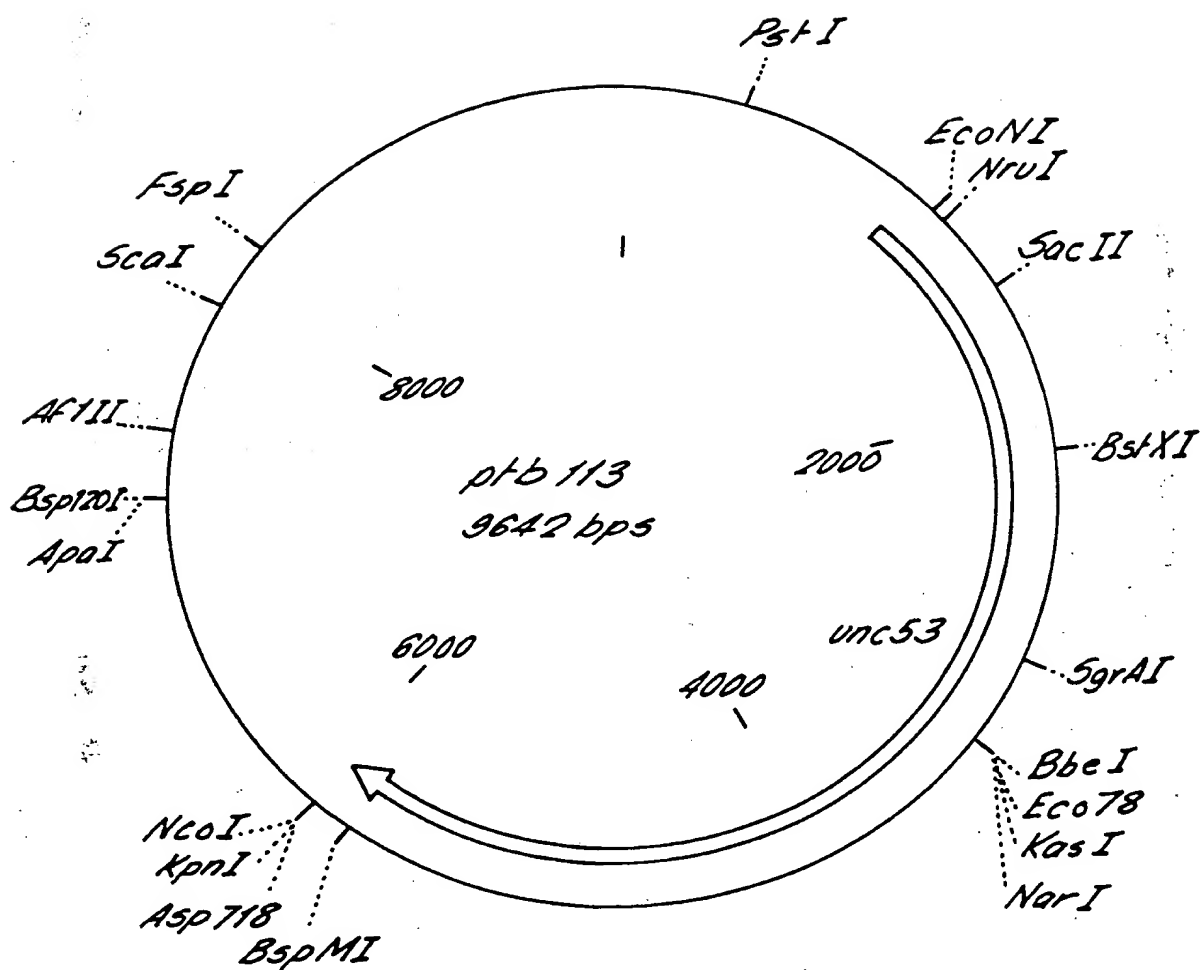
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*FIG. 37.*



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FIG. 38.



*92/99**FIG. 39.*

ATGACCATGA TTACGCCAAG CTTGTCTTCT TCTAAATTCC CATAAAATCC CGAAACTCCT	60
TCCCTCTATC TTCTTTTTCT TCTCGTTTTT AAATGTTTCT CTCTATCCCA TTCTCTCATC	120
AATTGAGTGG GATGAGGCTA TCTCTGCCTC TCTTCTGAAT CTCTGAACCA TCTTACATTA	180
CACTGTGGAT GACGAGCCCC ACAGGCTCCC TTGCATCAGA TACTGCCATT GGGGATGGCA	240
AAGAAGAGAG AAGGTATTGT GAGGATATAT TTTTCTAAGA AAAACGTTT GAAGAAAAGA	300
AGATGAAGAA GATCTGCTTG ATTCATTGCA CAAGTTAGAA GTAACAGGGG TCTATATTTT	360
GAAGAACTTA AAGGGAATGC AACTGAACAT AAAATTAAAC AAAGGGATTG AATCCTGCAG	420
TGAGTATTTT CGGTTTTTCA CTGGTTCTCT GTAAAAAGAG TAATGCAAAG GGCAAGTTAA	480
CTTAGGTCGT AAATGTATTG AATTTGCTTA AAATCTGAAG ATCTAGTGGT GAACCGTGGA	540
AGATTATCAA GAGGAGGCTG AAGATCTGTT TAAGAACCAT TAATCAAACCT GGTATTCTAT	600
TTTCACTGGT TGTATGTAAA CATTCTATCT TATTCCTTTT ATCACTGTTC TGCACTTTCC	660

FIG. 39 CONTINUED.

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TATAAAAAAA GTTGACCGAC CGTACTCTCT GAATTCATTT TTCCCGATCT TACCAACTCC	720
CGATCTATCT CTATCCCTGG TTTTCTCTC GTGCTCCAAT GGAATTCTTG AGACTTCCAC	780
TATCTTCTCT GGCACCTCC ACTACGCGTA GCGCTCTCTC GCTTCGTGTA TTCCCGGGAA	840
GCCGGTTCCC GTCTCTCCCG CCGCTGCCGC TGCCGCACAC AGCTTTACAC CTCGTAGAAT	900
CCCCAAAGAG GGGCGTGGCT TGCGGGTGCC AACATCCTCC TGCCGAGGAA GAAGCAGGCA	960
CTCATCACTC GCATCATCAA CCTCGGGATT GGCCAAAGGA CCCAAAGGTA TGTTTCGAAT	1020
GATACTAACA TAACATAGAA CATTTTCAGG AGGACCCTTG GCTAGAACTA GTGGATCCGA	1080
GCTCTCCCAT ATGACGACGT CAAATGTAGA ATTGATACCA ATCTACACGG ATTGGGCCAA	1140
TCGGCACCTT TCGAAGGGCA GCTTATCAAA GTCGATTAGG GATATTTCCA ATGATTTTCG	1200
CGACTATCGA CTGGTTTCTC AGCTTATTAA TGTGATCGTT CCGATCAACG AATTCTCGCC	1260
TGCATTCACG AAACGTTTGG CAAAATCAC ATCGAACCTG GATGGCCTCG AAACGTGTCT	1320
CGACTACCTG AAAAATCTGG GTCTCGACTG CTCGAACTC ACCAAAACCG ATATCGACAG	1380
CGGAAACTTG GGTGCAGTTC TCCAGCTGCT CTTCCTGCTC TCCACCTACA AGCAGAAGCT	1440
TCGGCAACTG AAAAAAGATC AGAAGAAATT GGAGCAACTA CCCACATCCA TTATGCCACC	1500
CGCGGTTTCT AAATTACCCT CGCCACGTGT CGCCACGTCA GCAACCGCTT CAGCAACTAA	1560
CCCAAATTCC AACTTTCCAC AAATGTCAAC ATCCAGGCTT CAGACTCCAC AGTCAAGAAT	1620
ATCGAAAATT GATTCATCAA AGATTGGTAT CAAGCCAAAG ACGTCTGGAC TTAAACCACC	1680
CTCATCATCA ACCACTTCAT CAAATAATAC AAATTCATTC CGTCCGTCGA GCCGTTGAG	1740
TGGCAATAAT AATGTTGGCT CGACGATATC CACATCTGCG AAGAGCTTAG AATCATCATC	1800
AACGTACAGC TCTATTTTGA ATCTAAACCG ACCTACCTCC CAACTCCAAA AACCTTCTAG	1860
ACCACAAACC CAGCTAGTTC GTGTTGCTAC AACTACAAAA ATCGGAAGCT CAAAGCTAGC	1920
CGCTCCGAAA GCCGTGAGCA CCCCCAAACT TGCTTCTGTG AAGACTATTG GAGCAAAACA	1980
AGAGCCCGAT AACAGCGGTG GTGGTGGTGG TGGAATGCTG AAATTAAAGT TATTCAGTAG	2040
CAAAAACCCA TCTTCCTCAT CGAATAGCCC ACAACCTACG AGAAAGGCGG CGGCGGTGCC	2100
TCAACAACAA ACTTTGTCTG AAATCGCTGC CCCAGTGAAA AGTGGCCTGA AGCCGCCGAC	2160
CAGTAAGCTG GGAAGTGCCA CGTCTATGTC GAAGCTTTGT ACGCCAAAAG TTTCTTACCG	2220
TAAACGGAC GCCCAATCA TATCTCAACA AGACTCGAAA CGATGCTCAA AGAGCAGTGA	2280
AGAAGAGTCC GGATACGCTG GATTCAACAG CACGTCGCCA ACGTCATCAT CGACGGAAGG	2340
TTCCCTAAGC ATGCATTCCA CATCTTCCAA GAGTTCAACG TCAGACGAAA AGTCTCCGTC	2400
ATCAGACGAT CTTACTCTTA ACGCCTCCAT CGTGACAGCT ATCAGACAGC CGATAGCCGC	2460
AACACCGGTT TCTCCAAATA TTATCAACAA GCCTGTTGAG GAAAAACCAA CACTGGCAGT	2520
GAAAGGAGTG AAAAGCACAG CGAAAAAAGA TCCACCTCCA GCTGTTCCGC CACGTGACAC	2580

FIG. 39 CONTINUED.

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CCAGCCAACA ATCGGAGTTG TTAGTCCAAT TATGGCACAT AAGAAGTTGA CAAATGACCC	2640
CGTGATATCT GAAAAACCAG AACCTGAAAA GCTCCAATCA ATGAGCATCG ACACGACGGA	2700
CGTTCCACCG CTTCCACCTC TAAAATCAGT TGTTCCACTT AAAATGACTT CAATCCGACA	2760
ACCACCAACG TACGATGTTC TTCTAAAACA AGGAAAAATC ACATCGCCTG TCAAGTCGTT	2820
TGGATATGAG CAGTCGTCCG CGTCTGAAGA CTCCATTGTG GCTCATGCGT CGGCTCAGGT	2880
GACTCCGCCG ACAAAAACTT CTGGTAATCA TTCGCTGGAG AGAAGGATGG GAAAGAATAA	2940
GACATCAGAA TCCAGCGGCT ACACCTCTGA CGCCGGTGTT GCGATGTGCG CCAAAATGAG	3000
GGAGAAGCTG AAAGAATACG ATGACATGAC TCGTCGAGCA CAGAACGGCT ATCCTGACAA	3060
CTTCGAAGAC AGTTCCTCCT TGTCGTCTGG AATATCCGAT AACACGAGC TCGACGACAT	3120
ATCCACGGAC GATTTGTCCG GAGTAGACAT GGCAACAGTC GCCTCCAAAC ATAGCGACTA	3180
TTCCCACTTT GTTCGCCATC CCACGTCTTC TTCTCAAAG CCCCGAGTCC CCAGTCGGTC	3240
CTCCACATCA GTCGATTCTC GATCTCGAGC AGAACAGGAG AATGTGTACA AACTTCTGTC	3300
CCAGTGCCGA ACGAGCCAAC GTGGCGCCGC TGCCACCTCA ACCTTCGGAC AACATTCGCT	3360
AAGATCCCCG GGATACTCAT CCTATTCTCC ACACTTATCA GTGTCAGCTG ATAAGGACAC	3420
AATGTCTATG CACTCACAGA CTAGTCGACG ACCTTCTTCA CAAAAACCAA GCTATTCAGG	3480
CCAATTTTCAT TCACTTGATC GTAAATGCCA CCTTCAAGAG TTCACATCCA CCGAGCACAG	3540
AATGGCGGCT CTCTTGAGCC CGAGACGGGT GCCGAACTCG ATGTCGAAAT ATGATTCTTC	3600
AGGATCCTAC TCGGCGCGTT CCCGAGGTGG AAGCTCTACT GGTATCTATG GAGAGACGTT	3660
CCAACTGCAC AGACTATCCG ATGAAAAATC CCCCGCACAT TCTGCCAAA GTGAGATGGG	3720
ATCCCAACTA TCACTGGCTA GCACGACAGC ATATGGATCT CTCAATGAGA AGTACGAACA	3780
TGCTATTCCG GACATGGCAC GTGACTTGGA GTGTTACAAG AACACTGTG ACTCACTAAC	3840
CAAGAAACAG GAGAACTATG GAGCATTGTT TGATCTTTTT GAGCAAAAGC TTAGAAAAT	3900
CACTCAACAC ATTGATCGAT CCAACTTGAA GCCTGAAGAG GCAATACGAT TCAGGCAGGA	3960
CATTGCTCAT TTGAGGGATA TTAGCAATCA TCTTGATCC AACTCAGCTC ATGCTAACGA	4020
AGGCGCTGGT GAGCTTCTTC GTCAACCATC TCTGGAATCA GTTGATCCC ATCGATCATC	4080
GATGTCATCG TCGTCGAAAA GCAGCAAGCA GGAGAAGATC AGCTTGAGCT CGTTTGCCAA	4140
GAACAAGAAG AGCTGGATCC GCTCCTCACT CTCCAAGTTC ACCAAGAAGA AGAACAAGAA	4200
CTACGACGAA GCACATATGC CATCAATTTC CGGATCTCAA GGAACTCTTG ACAACATTGA	4260
TGTGATTGAG TTGAAGCAAG AGCTCAAAGA ACGCGATAGT GCACTTTACG AAGTCCGCCT	4320
TGACAATCTG GATCGTGCCC GCGAAGTTGA TGTTCTGAGG GAGACAGTGA ACAAGTTGAA	4380
AACCGAGAAC AAGCAATTAA AGAAAGAAGT GGACAAACTC ACCAACGGTC CAGCCACTCG	4440
TGCTTCTTCC CGCGCCTCAA TTCCAGTTAT CTACGACGAT GAGCATGTCT ATGATGCAGC	4500

*FIG. 39 CONTINUED. 95/99*

GTGTAGCAGT	ACATCAGCTA	GTCAATCTTC	GAAACGATCC	TCTGGCTGCA	ACTCAATCAA	4560
GGTTACTGTA	AACGTGGACA	TCGCTGGAGA	AATCAGTTCG	ATCGTTAACC	CGGACAAAGA	4620
GATAATCGTA	GGATATCTTG	CCATGTCAAC	CAGTCAGTCA	TGCTGGAAAG	ACATTGATGT	4680
TTCTATTCTA	GGACTATTTG	AAGTCTACCT	ATCCAGAATT	GATGTGGAGC	ATCAACTTGG	4740
AATCGATGCT	CGTGATTCTA	TCCTTGGCTA	TCAAATTGGT	GAAC TTCGAC	GCGTCATTGG	4800
AGACTCCACA	ACCATGATAA	CCAGCCATCC	AACTGACATT	CTTACTTCCT	CAACTACAAT	4860
CCGAATGTTC	ATGCACGGTG	CCGCACAGAG	TCGCGTAGAC	AGTCTGGTCC	TTGATATGCT	4920
TCTTCCAAAG	CAAATGATTG	TCCAAC TCGT	CAAGTCAATT	TTGACAGAGA	GACGTCTGGT	4980
GTTAGCTGGA	GCAACTGGAA	TTGGAAAGAG	CAAAC TGGCG	AAGACCCTGG	CTGCTTATGT	5040
ATCTATTCTA	ACAAATCAAT	CCGAAGATAG	TATTGTTAAT	ATCAGCATTC	CTGAAAACAA	5100
TAAAGAAGAA	TTGCTTCAAG	TGGAACGACG	CCTGGAAGAG	ATCTTGAGAA	GCAAAGAATC	5160
ATGCATCGTA	ATTCTAGATA	ATATCCCAAA	GAATCGAATT	GCATTTGTTG	TATCCGTTTT	5220
TGCAAATGTC	CCACTTCAAA	ACAACGAAGG	TCCATTTGTA	GTATGCACAG	TCAACCGATA	5280
TCAAATCCCT	GAGCTTCAAA	TTCACCACAA	TTTCAAAATG	TCAGTAATGT	CGAATCGTCT	5340
CGAAGGATTC	ATCCTACGTT	ACCTCCGACG	ACGGGCGGTA	GAGGATGAGT	ATCGTCTAAC	5400
TGTACAGATG	CCATCAGAGC	TCTTCAAAAT	CATTGACTTC	TTCCCAATAG	CTCTTCAGGC	5460
CGTCAATAAT	TTTATTGAGA	AAACGAATTC	TGTTGATGTG	ACAGTTGGTC	CAAGAGCATG	5520
CTTGAAGTGT	CCTCTAACTG	TCGATGGATC	CCGTGAATGG	TTCAATCGAT	TGTGGAATGA	5580
GAAC TTCATT	CCATATTTGG	AACGTGTTGC	TAGAGATGGC	AAAAAACCT	TCGGTCGCTG	5640
CACTTCCTTC	GAGGATCCCA	CCGACATCGT	CTCTAAAAAA	TGGCCGTGGT	TCGATGGTGA	5700
AAACCCGGAG	AATGTGCTCA	AACGTCTTCA	ACTCCAAGAC	CTCGTCCCGT	CACCTGCCAA	5760
CTCATCCCGA	CAACACTTCA	ATCCCCCTCGA	GTCGTTGATC	CAATTGCATG	CTACCAAGCA	5820
TCAGACCATC	GACAACATTT	GAACAGAAGA	CTCTAATCTT	CTCTCGCCTC	TCCCCCGCTT	5880
TCCTTATCTT	CGTACCGGTA	CCATGGTATT	GATATCTGAG	CTCCGCATCG	GCCGCTGTCA	5940
TCAGATCGCC	ATCTCGCGCC	CGTGCCCTCTG	ACTTCTAAGT	CCAATTACTC	TTCAACATCC	6000
CTACATGCTC	TTTCTCCCTG	TGCTCCCAAC	CCCTATTTTT	GTTATTATCA	AAAAAACTTC	6060
TTCTTAATTT	CTTTGTTTTT	TAGCTTCTTT	TAAGTCACCT	CTAACAATGA	AATTGTGTAG	6120
ATTCAAAAAT	AGAATTAATT	CGTAATAAAA	AGTCGAAAAA	AATTGTGCTC	CCTCCCCCCA	6180
TTAATAATAA	TTCTATCCCA	AAATCTACAC	AATGTTCTGT	GTACACTTCT	TATGTTTTTT	6240
TTACTTCTGA	TAAATTTTTT	TTGAAACATC	ATAGAAAAAA	CCGCACACAA	AATACCTTAT	6300
CATATGTTAC	GTTTCAGTTT	ATGACCGCAA	TTTTTATTTT	TTCGCACGTC	TGGGCCCTCTC	6360
ATGACGTCAA	ATCATGCTCA	TCGTGAAAAA	GTTTTGGAGT	ATTTTTGGAA	TTTTTCAATC	6420

*FIG. 39 CONTINUED.**96/99*

AAGTGAAAGT TTATGAAATT AATTTTCCTG CTTTTGCTTT TTGGGGGTTT CCCCTATTGT	6480
TTGTCAAGAG TTTCGAGGAC GGCCTTTTTTC TTGCTAAAAT CACAAGTATT GATGAGCAGC	6540
ATGCAAGAAA GATCGGAAGA AGGTTTGGGT TTGAGGCTCA GTGGAAGGTG AGTAGAAGTT	6600
GATAATTTGA AAGTGGAGTA GTGTCTATGG GGTTTTTGCC TTAAATGACA GAATACATTC	6660
CCAATATACC AAACATAACT GTTTAAAATT AAACATTTTT CTAAATTTTA TATGATTTCT	6720
TTTAAATTTG CAAAAATTAC TTAAATTTGA ATTCCCGCGC AAATGAGTGA CTTCATTTTC	6780
TGCATTATTG TGTTTTCCGG CTATATTAAT AGGTATTTGT TTGTGTTTTT CTTTATTTTA	6840
TGATTGGAAC TCCAATTTGT AAATTTTCGA ACATATTTCC CTAAAGAAAA AATATGATTA	6900
ATCTGGAAAA ATTGGAAAAT TATTTTTCAA ATAAAAACA AAGAAAAAAA TGAAGAAAAA	6960
CCTATTAGTT TGGCCATAAA ACGCAAAAT GTCGAAAATG ACGTCACTCA TCTGCGCGGG	7020
AAATCAAGAA TAATTCGGCC TTTTTTATTT TTTTGGAAAA TCGTAAAACA TTTAGAAAAA	7080
TTTTTTAATA GTTATAGTGG GACTGTATTC TGTCATTTAG GGCAAAAGCC AGAGACGCTA	7140
CTCCACCGTT GGGGGATCCA CTAGTCGGCC GTACGGGCCC TTTCGTCTCG CGCGTTTCGG	7200
TGATGACGGT GAAAACCTCT GACACATGCA GCTCCCGGAG ACGGTCACAG CTTGTCTGTA	7260
AGCGGATGCC GGGAGCAGAC AAGCCCGTCA GGGCGCGTCA GCGGGTGTTG GCGGGTGTCG	7320
GGGCTGGCTT AACTATGCGG CATCAGAGCA GATTGTACTG AGAGTGCACC ATATGCGGTG	7380
TGAAATACCG CACAGATGCG TAAGGAGAAA ATACCGCATC AGGCGGCCTT AAGGGCCTCG	7440
TGATACGCTT ATTTTTATAG GTTAATGTCA TGATAATAAT GGTTTCTTAG ACGTCAGGTG	7500
GCACTTTTCG GGGAAATGTG CGCGGAACCC CTATTTGTTT ATTTTTCTAA ATACATTCAA	7560
ATATGTATCC GCTCATGAGA CAATAACCTT GATAAATGCT TCAATAATAT TGA AAAAGGA	7620
AGAGTATGAG TATTCAACAT TTCCGTGTCG CCCTTATTCC CTTTTTTGCG GCATTTTGCC	7680
TTCTGTGTTT TGCTCACCCA GAAACGCTGG TGAAAGTAAA AGATGCTGAA GATCAGTTGG	7740
GTGCACGAGT GGGTTACATC GAACTGGATC TCAACAGCGG TAAGATCCTT GAGAGTTTTC	7800
GCCCCGAAGA ACGTTTTCCA ATGATGAGCA CTTTTAAAGT TCTGCTATGT GGC GCGGTAT	7860
TATCCCGTAT TGACGCCGGG CAAGAGCAAC TCGGTCGCCG CATACTAT TCTCAGAATG	7920
ACTTGTTGA GTACTACCA GTCACAGAAA AGCATCTTAC GGATGGCATG ACAGTAAGAG	7980
AATTATGCAG TGCTGCCATA ACCATGAGTG ATAACACTGC GGCCAACTTA CTTCTGACAA	8040
CGATCGGAGG ACCGAAGGAG CTAACCGCTT TTTTGCACAA CATGGGGGAT CATGTAATC	8100
GCCTTGATCG TTGGGAACCG GAGCTGAATG AAGCCATACC AAACGACGAG CGTGACACCA	8160
CGATGCCTGT AGCAATGGCA ACAACGTTGC GCAAACTATT AACTGGCGAA CTACTTACTC	8220
TAGCTTCCCG GCAACAATTA ATAGACTGGA TGGAGGCGGA TAAAGTTGCA GGACCACTTC	8280
TGCGCTCGGC CCTTCCGGCT GGCTGGTTTA TTGCTGATAA ATCTGGAGCC GGTGAGCGTG	8340

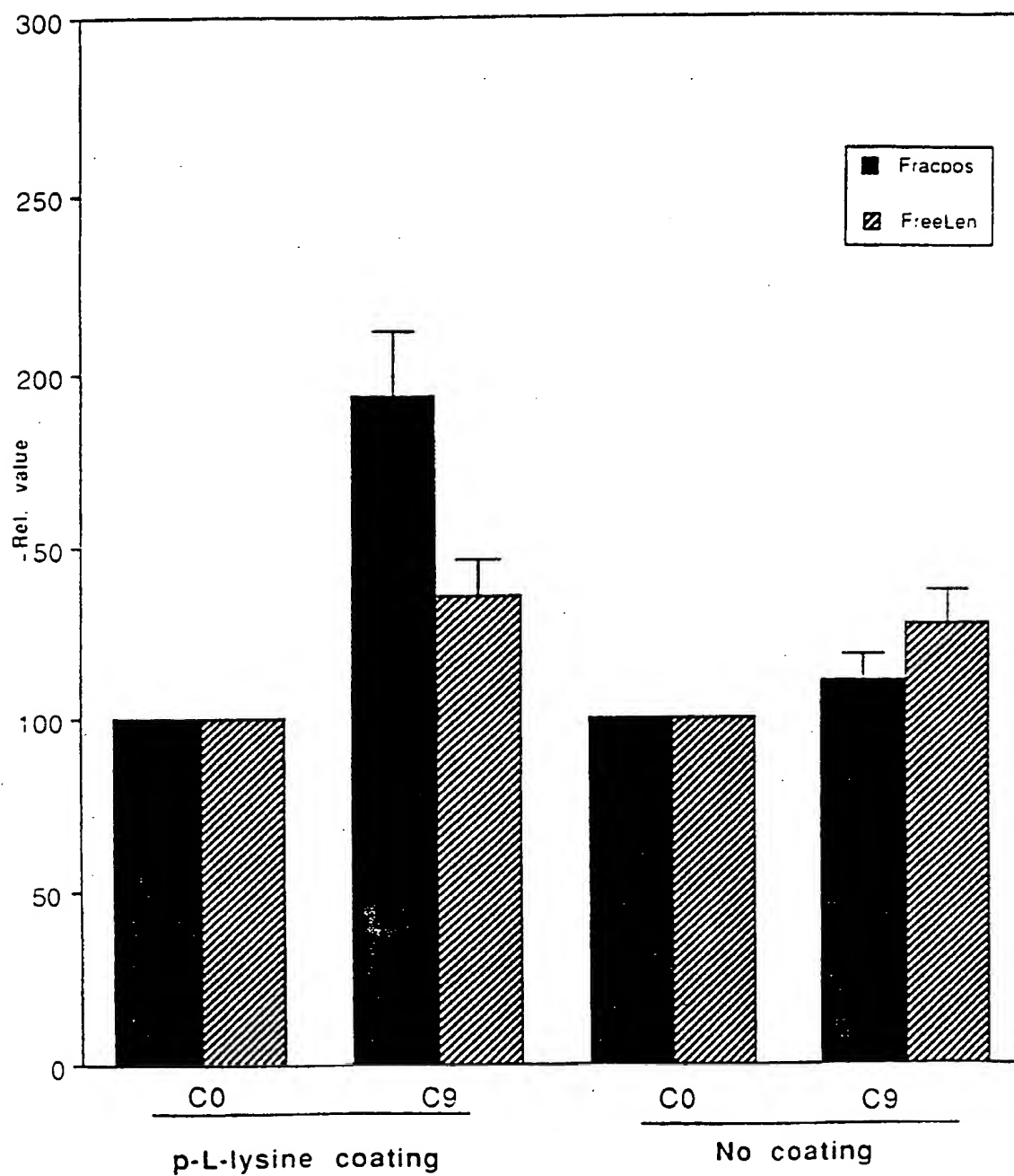


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## FIG. 39 CONTINUED.

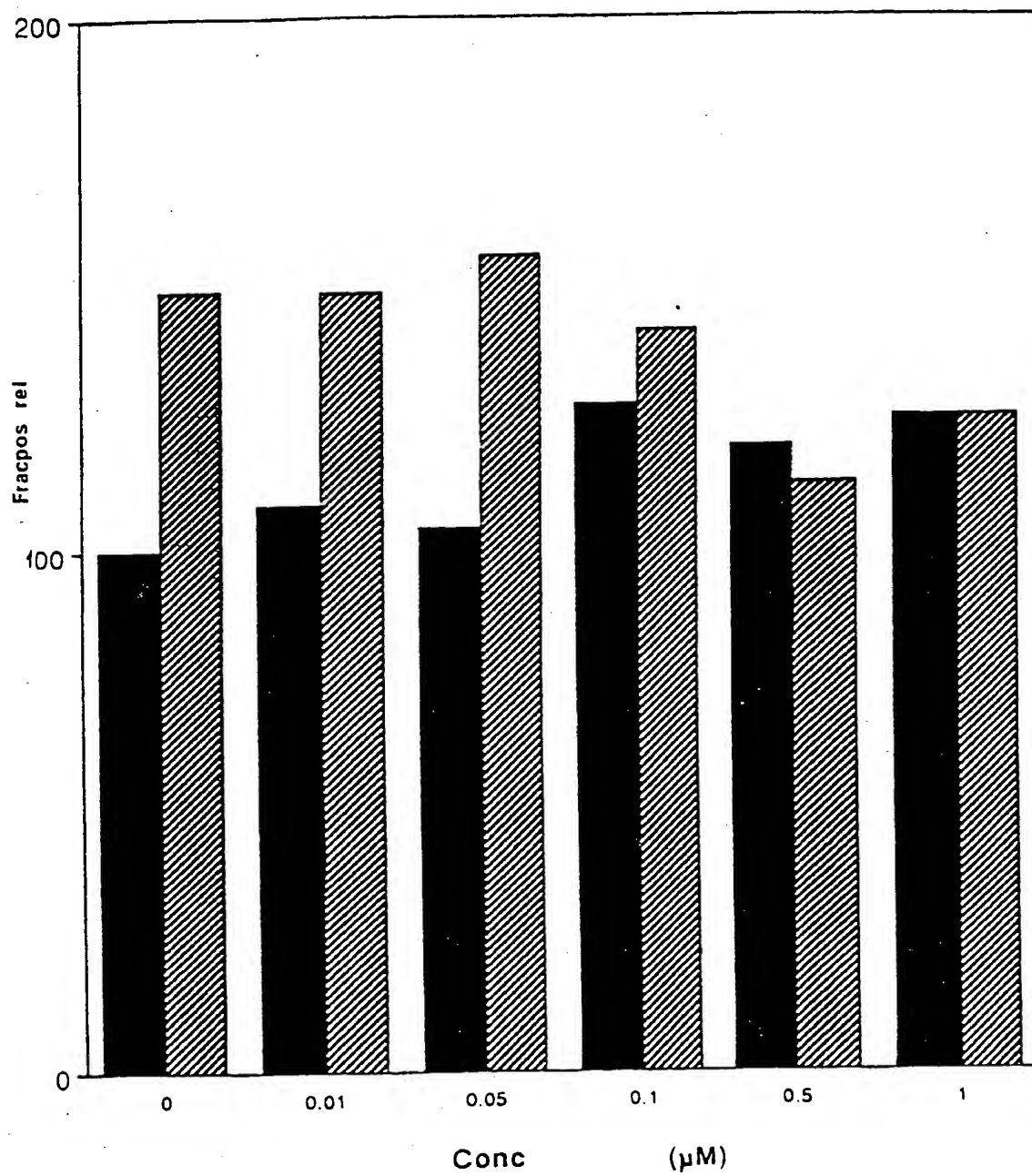
GGTCTCGCGG TATCATTGCA GCACTGGGGC CAGATGGTAA GCCCTCCCGT ATCGTAGTTA	8400
TCTACACGAC GGGGAGTCAG GCAACTATGG ATGAACGAAA TAGACAGATC GCTGAGATAG	8460
GTGCCTCACT GATTAAGCAT TGGTAACTGT CAGACCAAGT TTAATCATAT ATACTTTAGA	8520
TTGATTTAAA ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT TTTGATAATC	8580
TCATGACCAA AATCCCTTAA CGTGAGTTTT CGTTCCACTG AGCGTCAGAC CCCGTAGAAA	8640
AGATCAAAGG ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA	8700
AAAAACCACC GCTACCAGCG GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC	8760
CGAAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCTTCTA GTGTAGCCGT	8820
AGTTAGGCCA CCACTTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC	8880
TGTTACCAGT GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC	8940
GATAGTTACC GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA	9000
GCTTGAGCG AACGACCTAC ACCGAAGTGA GATACCTACA GCGTGAGCAT TGAGAAAGCG	9060
CCACGCTTCC CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG	9120
GAGAGCGCAC GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT	9180
TTCGCCACCT CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT	9240
GGAAAAACGC CAGCAACGCG GCCTTTTTAC GGTTCCTGGC CTTTGTCTGG CCTTTTGCTC	9300
ACATGTTCTT TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC GCCTTTGAGT	9360
GAGCTGATAC CGCTCGCCGC AGCCGAACGA CCGAGCGCAG CGAGTCAGTG AGCGAGGAAG	9420
CGGAAGAGCG CCCAATACGC AAACCGCCTC TCCCCGCGCG TTGGCCGATT CATTAAATGCA	9480
GCTGGCACGA CAGGTTTCCC GACTGGAAAG CGGGCAGTGA GCGCAACGCA ATTAATGTGA	9540
GTTAGCTCAC TCATTAGGCA CCCAGGCTT TACACTTTAT GCTTCCGGCT CGTATGTTGT	9600
GTGAATTGT GAGCGGATAA CAATTCACA CAGGAAACAG CT	9642

FIG. 40. 98/99



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FIG 41





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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/12, C07K 14/435, C12N 5/10, A01K 67/027, 67/033, A61K 38/17, A01H 5/00, C07K 16/18, C12N 5/26</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 96/38555</b> <b>(43) International Publication Date:</b> 5 December 1996 (05.12.96)
<b>(21) International Application Number:</b> PCT/EP96/02311 <b>(22) International Filing Date:</b> 31 May 1996 (31.05.96) <b>(30) Priority Data:</b> 9510944.3 31 May 1995 (31.05.95) <b>GB</b> <b>(71)(72) Applicants and Inventors:</b> BOGAERT, Thierry [BE/BE]; Voorstraat 36 bus 11, B-8500 Kortrijk (BE). STRINGHAM, Eve [CA/CA]; 9326-133 A Street, Surrey, British Columbia V3V 5R5 (CA). VANDEKERCKHOVE, Joel [BE/BE]; Rode Beukendreef 27, B-Loppem (BE). <b>(74) Agent:</b> BALDOCK, Sharon, Claire; Boulton Wade Tennant, 27 Fumival Street, London EC4A 1PQ (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> <b>(88) Date of publication of the international search report:</b> 30 January 1997 (30.01.97)
<b>(54) Title:</b> UNC-53 FROM C. ELEGANS AND ITS USES IN TESTING COMPOUNDS INVOLVED IN THE CONTROL OF CELL BEHAVIOUR AND PHARMACEUTICAL COMPOSITIONS  <b>(57) Abstract</b>  UNC-53 protein of <u>C. elegans</u> or its functional equivalent is identified as a signal transducer/integrator involved in controlling the rate and directionality of cell migration and/or cell shape. Nucleic acid sequences encoding UNC-53 protein or its functional equivalent, such as genomic or cDNA are used to transfect <u>C. elegans</u> or mammalian cell lines useful for identifying inhibitors or enhancers of the UNC-53 protein. Any of the inhibitors or enhancers identified or the UNC-53 protein itself or sequences encoding UNC-53 protein can be used in the preparation of medicament for treatment of neurological conditions such as Alzheimer's or Huntingdon's disease, peripheral neuropathies for inhibition of metastasis.		

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GA	Gabon			VN	Viet Nam

# INTERNATIONAL SEARCH REPORT

Inte onal Application No  
PCT/EP 96/02311

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/435 C12N5/10 A01K67/027 A01K67/033  
A01H5/00 A61K38/17 C07K16/18 C12N5/26

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N A01K A61K A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X  A	EMBL Database Entry CEF45E10 Accession number Z47810; 26 January 1995 XP002019188 & NATURE, vol. 368, no. 6466, 3 April 1994, LONDON GB, pages 32-38, R. WILSON ET AL.: "2.2 Mb of contiguous nucleotide sequence from chromosome III of C. elegans" see the whole document --- -/--	1-10

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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- \*&\* document member of the same patent family

Date of the actual completion of the international search

4 December 1996

Date of mailing of the international search report

13.12.96

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Authorized officer

Montero Lopez, B

# INTERNATIONAL SEARCH REPORT

Int. onal Application No  
PCT/EP 96/02311

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF NEUROSCIENCE 13 (10). 1993. 4254-4271. ISSN: 0270-6474, XP000612286 HEKIMI S ET AL: "Axonal guidance defects in a Caenorhabditis elegans mutant reveal cell-extrinsic determinants of neuronal morphology."	19,43
A	see abstract see page 4255, left-hand column, paragraph 2 - paragraph 3 see page 4267, right-hand column, paragraph 2 - page 4271, left-hand column, paragraph 3 -----	1-18, 20-42, 44-88